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## THE MORPHOGENESIS OF PITUITARY TUMORS INDUCED BY RADIOHYROIDECTOMY IN THE MOUSE AND THE EFFECTS OF THEIR TRANSPLANTATION ON THE PITUITARY BODY OF THE HOST \*

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Radiothyroidectomy leads to the formation of pituitary tumors in mice.<sup>1,2</sup> These growths are transplantable into athyroid mice<sup>3</sup> and in the course of grafting may acquire autonomy, whereafter they become established in normal recipients as well.<sup>4</sup> It has been shown that the transplanted neoplasms contain<sup>5,6</sup> and secrete<sup>4,7</sup> thyrotrophin, and that they possibly elaborate gonadotrophins.<sup>4,7</sup>

The present study deals with the changes which are encountered in the pituitary bodies of mice at various intervals after destruction of the thyroid gland and with the alterations which can be seen in the hypophyses of radiothyroidectomized and normal mice bearing transplanted pituitary tumors.

### MATERIALS AND METHODS

This study is based on the pituitary bodies of 124 C57BL mice of both sexes. The glands were fixed in Zenker's-formol or, more frequently, in a mixture of 9 parts susa fluid and 1 part saturated picric acid solution. They were embedded in paraffin and cut at 4 to 6  $\mu$  in the coronal plane. All tissues were stained by the following procedures: the modified Martins-Mallory method of Gude<sup>8</sup> or Gomori's trichrome,<sup>9</sup> the aldehyde fuchsin-trichrome technique of Halmi,<sup>10</sup> and the periodic acid-Schiff method, as applied by Purves and Griesbach.<sup>11</sup>

The following groups of mice were studied: (a) 15 normal males and females, (b) 90 radiothyroidectomized animals killed at intervals

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ranging from 10 to over 600 days after thyroid destruction, (c) 21 radiothyroidectomized hosts bearing dependent or autonomous\* transplanted tumors, (d) 6 normal hosts bearing autonomous grafted growths, and (e) 10 male mice killed at intervals of 30, 60, and 150 days after castration.

All radiothyroidectomized animals examined had received over 200  $\mu$ C. of  $I^{131}$ . This dose is sufficiently large to destroy the thyroid gland completely or at least to abolish the responsiveness of the few possibly surviving thyroid cells to thyrotrophin.<sup>7</sup>

## RESULTS

### *The Anterior Pituitary Lobe of Normal Mice*

Since no description of the mouse hypophysis based on the results of modern staining techniques has been published, the histologic appearance of the normal anterior lobe will be considered briefly.

The adenohypophysis of the mouse resembles that of the rat. It is made up of chromophobes and three types of chromophils: the acidophils, the beta cells, and the delta cells. The latter two classes together are the "basophils" of the older nomenclature. The acidophils form approximately 40 per cent of all cells of the anterior lobe. They are similar to the analogous cells of the rat in appearance and staining characteristics (periodic acid-Schiff's and aldehyde fuchsin, negative; orange G and chromotrope 2 R, stainable granules: Figs. 1, 2, and 8). A sex difference in the acidophils is not evident. The beta cells resemble closely those of the rat.<sup>12</sup> They are angular or even crescentic and most numerous in the central portions of the anterior lobe. Their granules are coarser than those of the acidophils and stain with both periodic acid-Schiff's and aldehyde fuchsin (Fig. 1). The number of these cells appears to be higher in males than in females, which is in agreement with the report by Wetzstein<sup>13</sup> who stained this cell type with kresofuchsin. The delta cells of the mouse are smaller than those of the rat and their highly periodic acid-Schiff's-positive, aldehyde fuchsin-negative granules are coarser (Fig. 6). They are most numerous in the isthmic median part of the anterior lobe which connects the bulkier lateral portions. They are not grouped in clusters around the portal vessels as in the rat.<sup>11</sup> The delta cells also may be somewhat less abundant in females than in males. Beta and delta cells together constitute less than 10 per cent of the cellular population of the anterior lobe.

\* For a discussion of these terms consult the article by Dr. Jacob Furth<sup>7</sup> in this issue.



*The Pituitary Body of Radiothyroidectomized Mice*

The changes subsequent to the administration of thyroid-destructive doses of  $I^{131}$  are confined to the anterior lobe.<sup>14</sup> Their temporal sequence can be conveniently considered under the following headings: (a) early thyroidectomy changes, (b) late thyroidectomy changes, (c) stage of appearance of microadenomas, and (d) stage of gross tumors.

*Early Thyroidectomy Changes.* The most drastic early thyroidectomy alterations occurred in the beta cells. As early as 10 days after radiothyroidectomy their aldehyde fuchsin-staining (beta) granules were completely absent and they were transformed into thyroidectomy cells<sup>14</sup> (Fig. 4), whose cytoplasm is aldehyde fuchsin negative and stains only faintly (if at all) with periodic acid-Schiff's stain. By 60 days after radiothyroidectomy, the thyroidectomy cells were fully developed and appeared to comprise up to one half of the volume of the anterior lobe. A comparison with the thyroidectomy cells of the rat<sup>11,15</sup> reveals the following differences: (a) the pattern of distribution in the mouse is more patchy; (b) there is a greater variability in the size of the cells in this species, some of them being truly gigantic and measuring up to 100  $\mu$  in diameter; (c) vacuolation is not the rule in the thyroidectomy cells of the mouse, or, if it occurs, it often remains confined to the emergence of small multiple vacuoles in the periphery of the cytoplasm (Fig. 5); (d) single or multiple hyaline cytoplasmic vacuoles may appear (Fig. 7), whose contents are slightly periodic acid-Schiff's-positive and are not coagulated by acid fixatives as in the rat; (e) periodic acid-Schiff's-positive colloid droplets<sup>11</sup> are less frequently encountered in the mouse thyroidectomy cells and never become as coarse as in the rat, and (f) mitotic figures are more frequently seen in the thyroidectomy cells of the mouse.

The delta cells showed no alterations 10 days after radiothyroidectomy. However, by 60 days after thyroid destruction they appeared distinctly enlarged and very heavily granulated (Fig. 8).

The acidophils, whose degranulation is so striking in radiothyroidectomized rats,<sup>16</sup> did not appear to be affected in the mouse in the early stages after thyroid destruction (Fig. 4).

*Late Thyroidectomy Changes.* In 120 to 150 days after radiothyroidectomy the pituitary bodies showed the full-blown thyroidectomy transformation of the beta cells as described, and a possible slight numerical decrease, but no conspicuous degranulation, of the acidophils (Fig. 7). The only marked difference from the glands showing "early thyroidectomy changes" was the presence of a large number of

cells which appeared to be hypertrophic delta cells. They were strongly periodic acid-Schiff's-positive, aldehyde fuchsin negative, displayed a prominent negative Golgi image, and frequently a single hyaline vacuole (Fig. 7). Their resemblance to the castration cells of the rat<sup>11</sup> is distinct.

*Stage of Appearance of Microadenomas.* In the pituitary bodies of mice sacrificed 6 to 10 months after radiothyroidectomy, adenomatous nodules were present. They varied in size from small clusters of cells, which could not be differentiated reliably from simple aggregations of thyroidectomy cells, to adenomas occupying one half of the anterior lobe. Although the cells making up these nodules had a cytoplasm which was "chromophobic," i.e., periodic acid-Schiff's and aldehyde fuchsin negative and apparently devoid of granules, they were much larger than the chromophobes of the normal pituitary body, usually had distinct boundaries, and generally resembled non-vacuolated thyroidectomy cells (Fig. 11). The nodules were more or less distinctly demarcated from their surroundings (Figs. 10 and 11) and sometimes compressed the adjacent glandular tissue. Occasional nodules were made up of extensively vacuolated cells which were in every respect similar to the thyroidectomy cells of the rat (Fig. 9). Mitotic figures were infrequent in the adenomas.

In the parenchyma between the nodules the described thyroidectomy changes were seen. Acidophils were sometimes slightly diminished in numbers but usually abundant (Fig. 9). Prominent and enlarged delta cells were not encountered.

*Stage of Gross Tumors.* The mice with greatly enlarged pituitary bodies (greater than 5 mm. in diameter) had received I<sup>131</sup> 10 months or more prior to necropsy. In all of these animals the normal structure of the pituitary body was effaced. Occasionally greatly compressed remnants of the original parenchyma, still containing well granulated acidophils, could be found peripherally. The bulk of the organ was occupied by neoplastic tissue made up of periodic acid-Schiff's and aldehyde fuchsin negative cells which either resembled smaller thyroidectomy cells (Fig. 12) or had less distinct cell borders (Fig. 16). In one example many cells of the growth showed advanced vacuolation reminiscent of the classical thyroidectomy cell of the rat (Fig. 15). Further features of these tumors were follicle-like formations (Fig. 13), cells with periodic acid-Schiff's-positive granules resembling delta cells interspersed with the predominant "chromophobic" cells (Fig. 13), periodic acid-Schiff's reactive intranuclear inclusions (Fig. 14), and widespread hemorrhages (Fig. 12). The tumor cells were usually quite uniform and but few mitotic figures were encountered. The

histologic features of these primary pituitary growths are similar to those of transplanted tumors of dependent strains.<sup>7</sup>

The relationship between the gross pituitary tumors and the microscopic adenomas seen in earlier stages is not altogether clear. It is likely that the former are the result of further growth and confluence of multiple circumscribed micro-adenomas.

*Effects of Grafted Dependent or Autonomous Tumors on the Pituitary Bodies of Radiothyroidectomized Hosts*

The pituitary bodies examined were those of hosts which had been radiothyroidectomized 150 to 300 days prior to necropsy and were bearing grafted tumors measuring over 2 cm. across. All showed thyroidectomy changes and most of them contained adenomas. The following findings deserve emphasis: (a) Whereas beta granules were lacking from the pituitary bodies of radiothyroidectomized mice which had not been grafted, in the tumor hosts, typical, fully granulated beta cells often could be seen among the thyroidectomy cells or some of the latter contained beta granules in the outer portion of their cytoplasm (Fig. 17). Occasionally the cells of adenomatous nodules were heavily charged with beta granules. (b) Delta cells were either completely absent from these pituitary glands or, at least, were less prominent than in normal glands.

The findings in 2 of the 7 female mice are of special interest. In one, which was bearing a dependent graft measuring 3 cm. across, one half of the only moderately enlarged anterior lobe was occupied by a growth which showed histologic criteria of malignancy, viz., tremendous nuclear polymorphism, multinucleated cells (Fig. 20), a great number of mitotic figures, many of which were abnormal (Fig. 19), and infiltrative growth as indicated by the presence of acidophils amidst the tumor cells (Fig. 20) and by the invasion of the sellar periosteum by the neoplasm. The growth was histologically similar to autonomous transplanted tumors.<sup>7</sup> In the second mouse, which was the host of an autonomous tumor, a neoplastic growth showing numerous mitotic figures was found to be invading a greatly dilated sinusoid (Fig. 18). The possibility that this was a metastasis from the grafted growth cannot be excluded.

*Effects of Grafted Autonomous Tumors on the Pituitary Bodies of Normal Hosts*

The most striking change in the pituitary bodies of normal hosts bearing large autonomous grafts was the diminution in the size of the acidophils (Fig. 3). Furthermore, the beta cells were definitely and

sometimes almost completely degranulated and were variably reduced in size. The delta cells appeared somewhat sparser than in glands of normal controls.

#### DISCUSSION

There is good evidence that the beta cells of the rat, also called "thyrotrophs" by Purves and Griesbach,<sup>11</sup> produce thyrotrophin,<sup>17,18</sup> since they give rise to thyroidectomy cells in states of thyroid hormone deficiency<sup>17</sup> and regress under the effect of thyroxin.<sup>17,19</sup> The findings reported in this paper indicate that the beta cells of the mouse play the same physiologic rôle. Their degranulation and transformation into thyroidectomy cells constitute the most striking response of the pituitary body to radiothyroidectomy.\* There is little doubt that the multifocal adenomas and the finally developing gross tumors also are composed of more or less typical or dedifferentiated thyroidectomy cells. The relationship of these to beta cells is emphasized by the reappearance of beta granules in cells composing adenomas in the pituitary bodies of radiothyroidectomized hosts bearing grafted tumors. This finding brings to mind the regranulation of the thyroidectomy cells of the athyroid rat in response to incomplete replacement therapy with thyroxin.<sup>18</sup> It is indeed possible that extrathyroidal synthesis of thyroxin is stepped up in tumor-bearing radiothyroidectomized mice under the influence of the large amounts of thyrotrophin secreted by the tumor and that this accounts for the partial regranulation of the thyroidectomy cells. The regressive changes of the beta cells in normal hosts grafted with autonomous tumors are in all probability equivalent to the beta cell alterations of rats given thyroxin, since these mice invariably have greatly enlarged and presumably overactive thyroid glands.<sup>4,7</sup>

Although indirect evidence indicates that the beta granules of the rat's pituitary body contain thyrotrophin,<sup>17,19</sup> a high thyrotrophin content was found in transplanted radiothyroidectomy-induced pituitary tumors<sup>5,6</sup> which are completely devoid of beta granulation. It is probable, however, that the concentration of thyrotrophin is much higher in normal beta cells than in the cells of the tumor, and Purves and Griesbach<sup>17</sup> have pointed out that the total absence of beta granules from the pituitary body of the athyroid rat is compatible with the presence of demonstrable, albeit diminished, thyrotrophin stores in the gland.

The morphologic and histochemical similarities between the delta cells of the mouse pituitary gland and the "gonadotrophs" of the rat

\* Similar changes were observed in C<sub>3</sub>H mice fed propylthiouracil for 3 weeks.

are definite. However, their physiologic kinship remains to be established. The well known castration changes of the pituitary body of the rat, which are due to a spectacular hyperplasia, hypertrophy, and progressive vacuolization of heavily granulated delta cells or "gonadotrophs,"<sup>11,12</sup> cannot be reproduced in the mouse. In fact, there was but little change in the number of delta cells in the pituitary bodies of castrated male mice examined at intervals of 30, 60, and 150 days after castration, small "signet ring" cells were only occasionally seen, and the periodic acid-Schiff staining granules were diminished rather than increased. Thus it is not possible to relate the changes in the delta cells of radiothyroidectomized mice, which appear to culminate in the "late thyroidectomy" stage (Fig. 7), to gonadal damage, especially since they occur in both sexes, whereas the administration of thyroid-destructive doses of  $I^{131}$  causes histologic damage to the ovaries but not to the testes.<sup>2</sup> If it is assumed that the delta cells of the mouse are gonadotrophic in function, their apparent regressive changes in mice bearing tumor grafts are in better agreement with the idea that gonadal stimulation in these animals<sup>4,7</sup> is due to a secretion of the tumor than with the concept that it is the sequel of enhanced gonadotrophin output by the pituitary body.

The fact that radiothyroidectomy has little effect on the acidophils of the mouse deserves comment. In the rat, total degranulation of the acidophils ensues if hypothyroidism is severe.<sup>16,20,21</sup> Since it is probable that thyroid function was more or less completely abolished in the radiothyroidectomized mice of this study,<sup>7</sup> the persistence of the acidophils indicates either that a substantial amount of extrathyroidal thyroxin is formed in the mouse or that the acidophils in this species do not depend on thyroid hormone for their maintenance. The reduced size of the acidophils in normal hosts bearing autonomous grafts appears to be the result of hyperthyroidism, since similar changes have been observed in thyroxin-treated rats.<sup>19</sup>

The occurrence of highly anaplastic, invasive growths in the pituitary bodies of two grafted radiothyroidectomized female mice is of great interest. Similar changes were not observed in the 14 male hosts studied, nor in athyroid mice not bearing grafts. This may be purely fortuitous, but it is challenging to consider that these growths might have been due to the combination of the effects of thyroid destruction with a possibly gonad-mediated influence of tumor products on the pituitary body. In view of the known oncogenic properties of estrogens, their effect on the hypophysis of radiothyroidectomized mice should be in-



vestigated. It also remains to be seen whether such tumors as were encountered in the two female hosts would prove to be autonomous and hormonally active upon transplantation.

#### SUMMARY AND CONCLUSIONS

The pituitary body of the mouse possesses three types of chromophil cells: acidophils, beta cells, and delta cells.

Radiothyroidectomy leads to a rapid transformation of the beta cells into thyroidectomy cells through degranulation, hyperplasia, and hypertrophy. After 6 months, multifocal adenomas made up of typical or dedifferentiated thyroidectomy cells appear. Ten months after thyroid destruction gross tumors composed of similar but usually even less differentiated cells arise in the pituitary body. A transient hypertrophy of the delta cells occurs prior to the appearance of the adenomas. There is little change in the acidophils until they are "crowded out" by the tumor.

Radiothyroidectomized hosts bearing grafted dependent or autonomous pituitary tumors may show a partial reappearance of beta granules in the thyroidectomy cells and a regression of the delta cells. In two of seven female hosts pituitary growths exhibiting histologic signs suggestive of malignancy were encountered.

Autonomous tumors grafted into normal hosts depress all three types of chromophil cells, especially the acidophils.

The significance of these findings in the histophysiology of the pituitary body of the mouse is discussed.

#### REFERENCES

1. Gorbman, A. Tumorous growth in the pituitary and trachea following radio-toxic dosages of  $I^{131}$ . *Proc. Soc. Exper. Biol. & Med.*, 1949, 71, 237-240.
2. Gorbman, A. Functional and structural changes consequent to high dosages of radioactive iodine. *J. Clin. Endocrinol.*, 1950, 10, 1177-1191.
3. Furth, J., and Burnett, W. T., Jr. Hormone-secreting transplantable neoplasms of the pituitary induced by  $I^{131}$ . *Proc. Soc. Exper. Biol. & Med.*, 1951, 78, 222-224.
4. Furth, J., Gadsden, E. L., and Burnett, W. T., Jr. Autonomous transplantable pituitary tumors arising in growths dependent on absence of the thyroid gland. *Proc. Soc. Exper. Biol. & Med.*, 1952, 80, 4-7.
5. Furth, J., Burnett, W. T., Jr., and Gadsden, E. L. Quantitative relationship between thyroid function and growth of pituitary tumors secreting TSH. *Cancer Research*, 1953, 13, 298-307, and unpublished data.
6. Halmi, N. S., Spirtos, B. N., Bogdanove, E. M., and Lipner, H. J. A study of various influences on the iodide concentrating mechanism of the rat thyroid. *Endocrinology*, 1953, 52, 19-32.
7. Furth, J. Morphologic changes associated with thyrotrophin-secreting pituitary tumors. *Am. J. Path.*, 1954, 30, 421-463.



8. Gude, W. D. Modified Martins-Mallory stain for mouse pituitary gland. *Stain Technol.*, 1953, 28, 161-162.
9. Gomori, G. A rapid one-step trichrome stain. *Am. J. Clin. Path.*, 1950, 20, 661-664.
10. Halmi, N. S. Differentiation of two types of basophils in the adenohypophysis of the rat and the mouse. *Stain Technol.*, 1952, 27, 61-64.
11. Purves, H. D., and Griesbach, W. E. The site of thyrotrophin and gonadotrophin production in the rat pituitary studied by McManus-Hotchkiss staining for glycoprotein. *Endocrinology*, 1951, 49, 244-264.
12. Halmi, N. S. Two types of basophils in the anterior pituitary of the rat and their respective cytophysiological significance. *Endocrinology*, 1950, 47, 289-299.
13. Wetzstein, R. Die Hypophyse der Maus bei langdauernder peroraler Verabreichung geringer Mengen von Follikelhormon; ihr besonderes Verhalten bei gleichzeitig auftretender Degeneration der Nebennieren. *Arch. f. Entwicklungsmech. d. Organ.*, 1940, 140, 379-408.
14. Goldberg, R. C., and Chaikoff, I. L. On the nature of the hypertrophied pituitary gland induced in the mouse by  $I^{131}$  injections, and the mechanism of its development. *Endocrinology*, 1951, 48, 1-5.
15. Reese, J. D., Koneff, A. A., and Wainman, P. Cytological differences between castration and thyroidectomy basophils in the rat hypophysis. *Essays in Biol.*, 1943, pp. 471-485.
16. Goldberg, R. C., and Chaikoff, I. L. The cytological changes that occur in the anterior pituitary glands of rats injected with various doses of  $I^{131}$  and their significance in the estimation of thyroid function. *Endocrinology*, 1950, 46, 91-104.
17. Purves, H. D., and Griesbach, W. E. The significance of the Gomori staining of the basophils of the rat pituitary. *Endocrinology*, 1951, 49, 652-662.
18. Halmi, N. S. Two types of basophils in the rat pituitary; "thyrotrophs" and "gonadotrophs" vs. beta and delta cells. *Endocrinology*, 1952, 50, 140-142.
19. Halmi, N. S. The effects of graded doses of thyroxin on the anterior pituitary of hypothyroid male albino rats. *Anat. Rec.*, 1952, 112, 17-35.
20. Lebedewa, N. S. Der histophysiologische Effekt der Thyreoidektomie im Hypophysenvorderlappen der Ratte. *Arch. f. exper. Path. u. Pharmacol.*, 1936, 183, 15-29.
21. Purves, H. D., and Griesbach, W. E. Observations on the acidophil cell changes in the pituitary in thyroxine deficiency states. I. Acidophil degranulation in relation to goitrogenic agents and extrathyroidal thyroxine synthesis. *Brit. J. Exper. Path.*, 1946, 27, 170-179.

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[ Illustrations follow ]

## LEGENDS FOR FIGURES

- FIG. 1. Normal mouse pituitary body. Arrows point to beta cells. Cytoplasm of acidophils is lighter gray. Aldehyde fuchsin-trichrome stain.  $\times 1280$ .
- FIG. 2. Normal mouse pituitary body, showing acidophils, chromophobes, and one "basophil" (probably a delta cell) in the center (arrow). Gomori's trichrome stain.  $\times 640$ .
- FIG. 3. Pituitary body of normal host bearing an autonomous graft. Size of acidophils is strikingly reduced. For comparison with Figure 2. Gomori's trichrome stain.  $\times 640$ .
- FIG. 4. Mouse pituitary body 23 days after radiothyroidectomy. Acidophils are normal. Large thyroidectomy cells are prominent. Gomori's trichrome stain.  $\times 640$ .





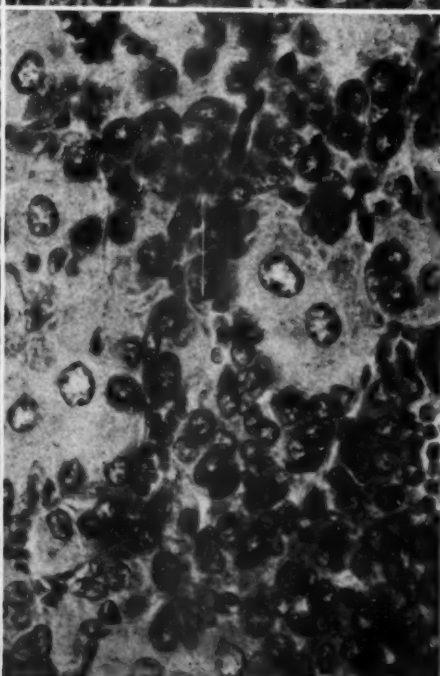
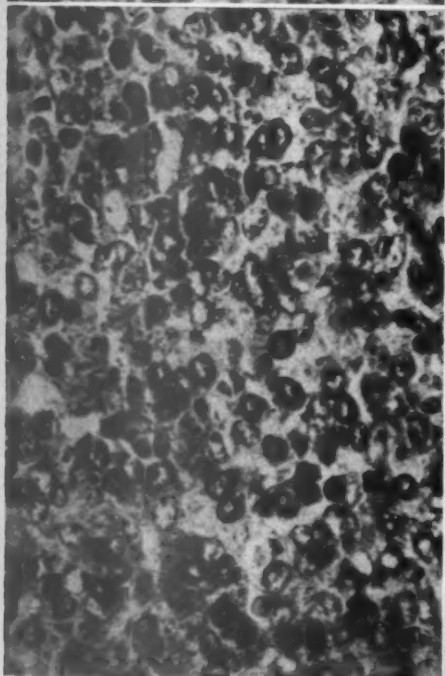
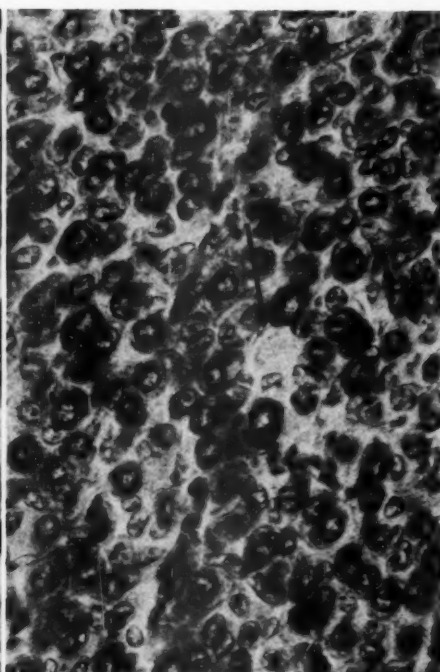
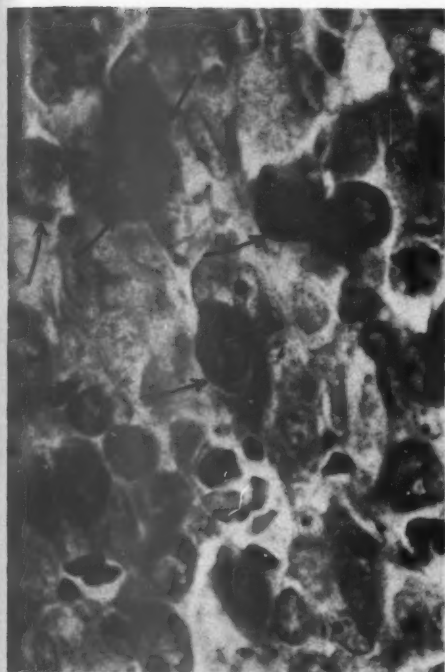


FIG. 5. From the same section as Figure 4. There are multiple peripheral vacuoles in the cytoplasm of a thyroidectomy cell. Acidophils are fully granulated. Gomori's trichrome stain.  $\times 1280$ .

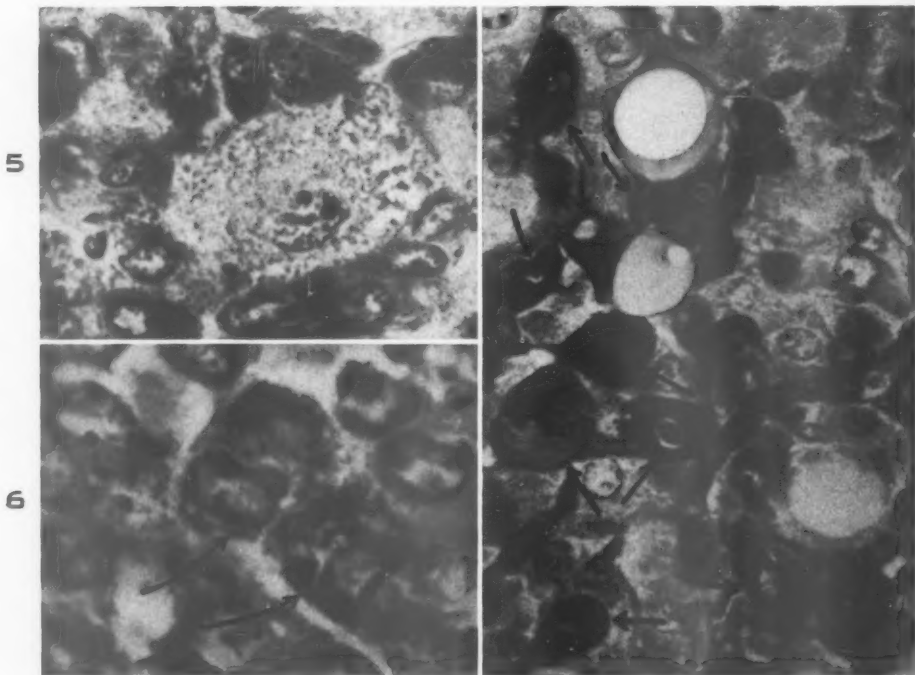
FIG. 6. Normal mouse pituitary body. Arrows point to delta cells. There are coarse, periodic acid-Schiff's-positive granules. Periodic acid-Schiff's and hemalum stains.  $\times 1700$ .

FIG. 7. Pituitary body 136 days after radiothyroidectomy. Many pale staining thyroidectomy cells, some of them with large hyaline vacuoles. Arrows point to hypertrophic, heavily granulated delta cells. Of note are negative Golgi images and a hyaline vacuole in one cell. Smaller cells with gray cytoplasm are acidophils. Martins-Mallory stain.  $\times 640$ .

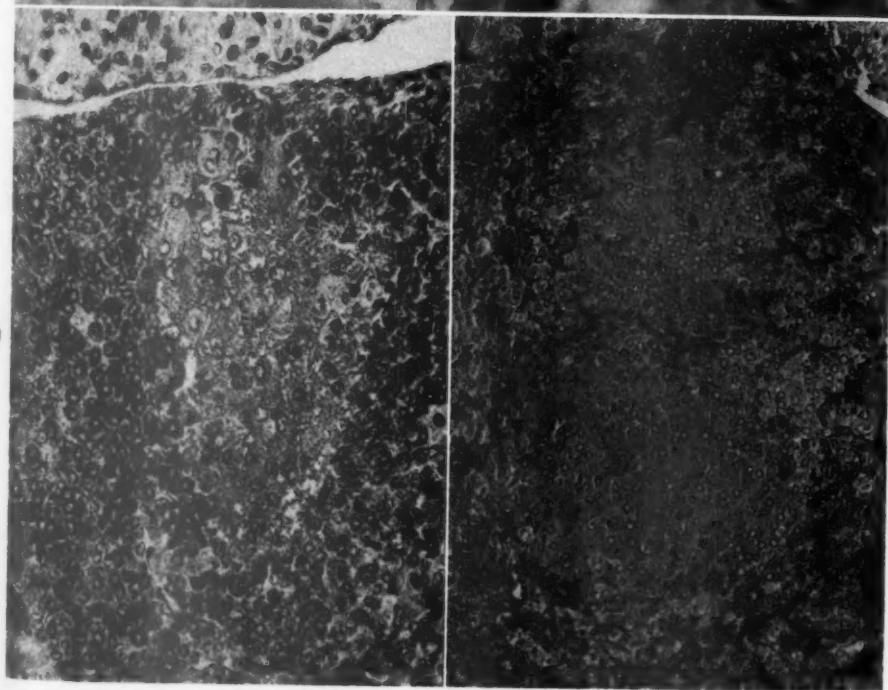
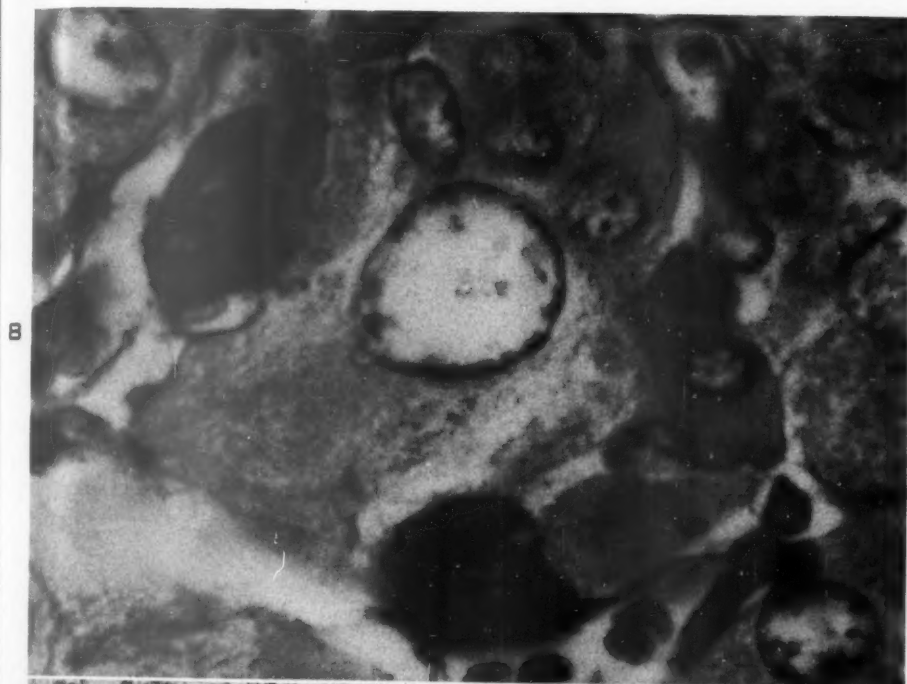
FIG. 8. Mouse pituitary body 60 days after radiothyroidectomy. Large thyroidectomy cell surrounded by chromophobes, acidophils (with homogeneous, gray cytoplasm) and two enlarged, heavily granulated delta cells. For comparison with Figure 6. Periodic acid-Schiff's and hemalum stains.  $\times 1700$ .

FIG. 9. Adenomatous nodule composed of thyroidectomy cells showing multiple cytoplasmic vacuoles (170 days after radiothyroidectomy). Many acidophils in the parenchyma surrounding a nodule. Hypophysial cleft and intermediate lobe in upper part of the field. Gomori's trichrome stain.  $\times 280$ .

FIG. 10. Adenomatous nodule (276 days after  $I^{131}$  administration) composed of medium-sized, non-vacuolated thyroidectomy cells. Compression of surrounding tissue is not evident. Martins-Mallory stain.  $\times 140$ .







- FIG. 11. Pituitary body of mouse sacrificed 247 days after radiothyroidectomy. Adenomatous nodule (upper part of field). Of note is the distinct boundary. The adenoma is composed of fairly large, non-vacuolated thyroidectomy cells. Gomori's trichrome stain.  $\times 280$ .
- FIG. 12. Gross pituitary tumor (465 days after radiothyroidectomy) composed of medium-sized thyroidectomy-like cells. Patchy hemorrhage may be noted. Martins-Mallory stain.  $\times 280$ .
- FIG. 13. Follicle-like structure and cell with periodic acid-Schiff's-positive granulation (arrow) in gross pituitary tumor (642 days after radiothyroidectomy). Periodic acid-Schiff's and hemalum stains.  $\times 640$ .
- FIG. 14. From the same slide as Figure 13. Periodic acid-Schiff's-positive intranuclear inclusions. Periodic acid-Schiff's and hemalum stains.  $\times 1280$ .
- FIG. 15. From the same slide as Figure 13. Large number of thyroidectomy-type cells showing advanced vacuolation. Periodic acid-Schiff's and hemalum stains.  $\times 280$ .





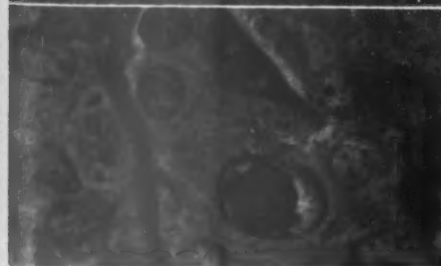
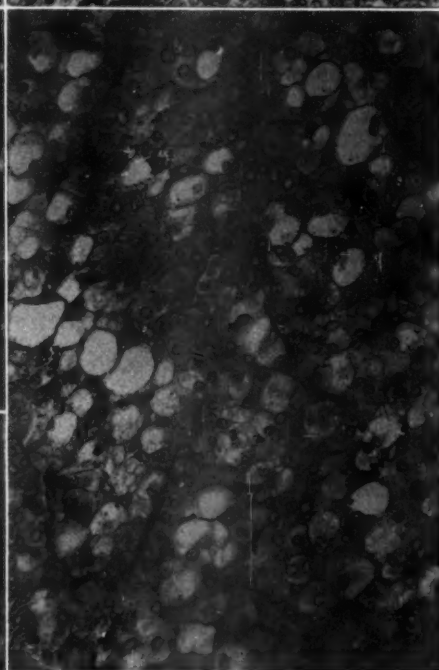
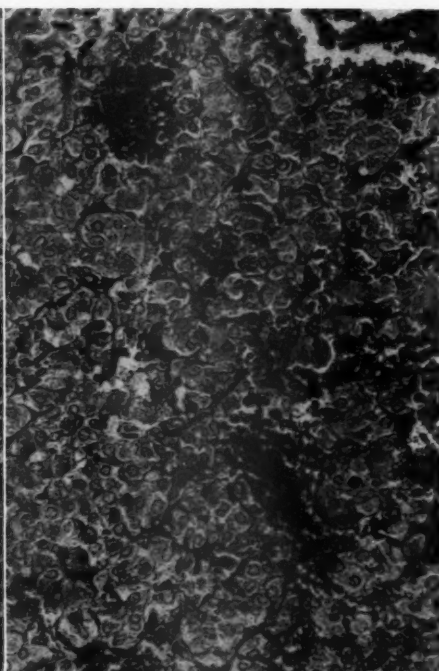
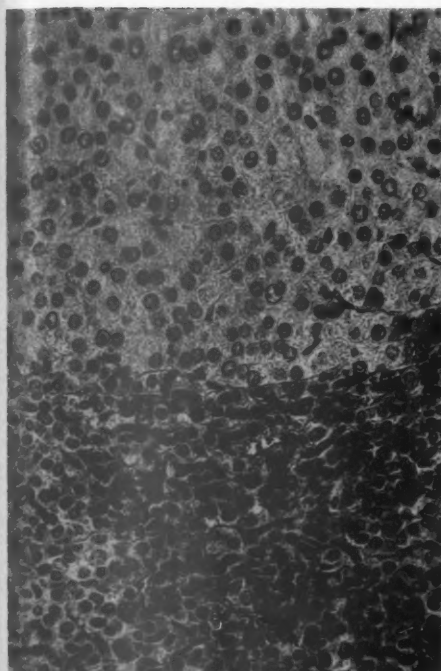


FIG. 16. Grossly evident pituitary tumor made up of uniform "chromophobic" cells without distinct boundaries (437 days after radiothyroidectomy). Martins-Mallory stain.  $\times 640$ .

FIG. 17. From pituitary body of radiothyroidectomized host bearing an autonomous graft (140 days after  $I^{131}$  administration). Fully granulated beta cells (upper arrow) and partially regranulated thyroidectomy cell (lower arrow) among typical degranulated thyroidectomy cells. Aldehyde fuchsin-trichrome stain.  $\times 640$ .

FIG. 18. Pituitary body of radiothyroidectomized female host bearing an autonomous graft (140 days after the administration of  $I^{131}$ ). Somewhat anaplastic tumor tissue (mitotic figures may be noted) invading a greatly dilated sinusoid (distinct basement membrane, erythrocytes in lumen). Surrounding parenchyma shows thyroidectomy changes. Periodic acid-Schiff's and hemalum stains.  $\times 280$ .

FIG. 19. Atypical growth in pituitary body of radiothyroidectomy female host bearing a grafted dependent tumor (213 days after  $I^{131}$  administration). Great nuclear polymorphism and many abnormal mitotic figures are seen. Gomori's trichrome stain.  $\times 640$ .

FIG. 20. From the same section as Figure 19. Arrows point to acidophils surrounded by tumor cells. There are several multinucleated cells. Gomori's trichrome stain.  $\times 640$ .

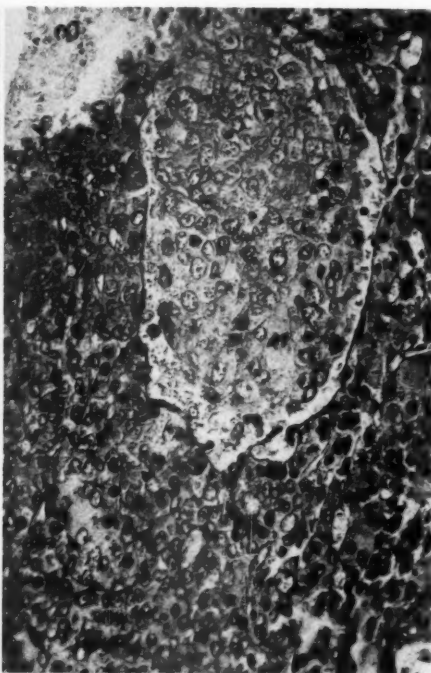
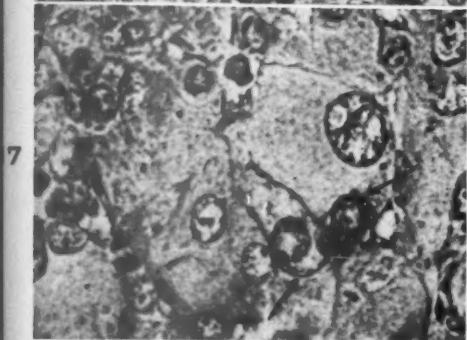
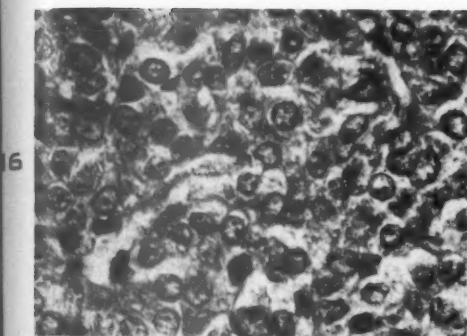




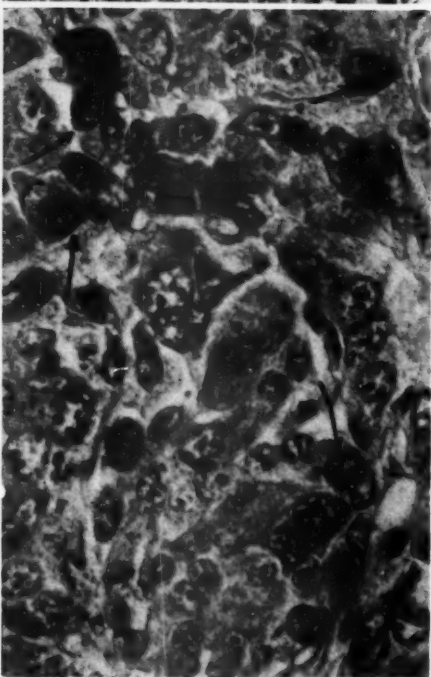
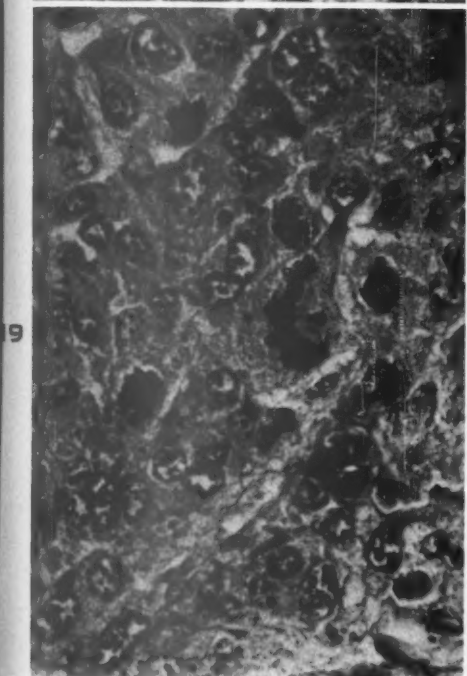
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## MORPHOLOGIC CHANGES ASSOCIATED WITH THYROTROPHIN-SECRETING PITUITARY TUMORS\*

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Pituitary tumors can be induced readily in mice by radiothyroidectomy.<sup>1</sup> Transplantation of these growths is highly successful in mice in which thyroid function is depressed, but not in normal mice.<sup>2</sup> In the course of successful transplantation many of these conditioned pituitary growths acquire autonomy, can be grafted in normal mice, and after a few passages in normal hosts they grow even better in normal than in radiothyroidectomized animals.<sup>3</sup> Administration of thyroid hormone to radiothyroidectomized mice prevents the induction of such tumors<sup>4</sup> and retards or prevents the growth of grafted dependent tumors.<sup>5</sup> Morphologic evidence will be presented here to indicate that these tumors secrete large quantities of thyrotrophin. Changes in tumor-bearing hosts indicate secretion of gonadotrophin in some hosts. Acquisition of autonomy is associated with characteristic morphologic changes in tumor cells and in stromal reaction to them. The main purpose of this study is to describe and illustrate the remarkable morphologic changes observed in the tumors and tumor-bearing hosts; their pathogenesis remains to be studied.

### MATERIAL AND METHODS

The induction and transplantation of these pituitary tumors have been described.<sup>2,3,6</sup> Seven tumors induced in C57 black mice have been carried in serial passages. Three of these have given rise to autonomous growths in the course of subpassages.

Normal mice, 6 to 10 weeks of age, were injected subcutaneously with 100 to 400  $\mu$ c. of carrier-free  $I^{131}$  in 2 cc. of physiologic saline solution. These doses destroy the thyroid glands of mice kept on a standard diet consisting of Purina chow *ad libitum* supplemented once weekly with carrots or lettuce. Mice 2 weeks old were radiothyroidectomized with 50  $\mu$ c. of  $I^{131}$ . The tumor grafts were made 2 to 5 weeks

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following administration of  $I^{131}$ . The tumors were transplanted by injecting tumor fragments in physiologic saline solution into the thigh muscles of normal and radiothyroidectomized mice. All animals were necropsied. Microscopic examinations were made whenever gross inspection was inadequate to evaluate the changes, and for special studies. In addition to the common histologic staining techniques, Gude's<sup>7</sup> combination of Martins and Mallory's trichrome stain, the periodic acid-Schiff method, and the aldehyde fuchsin technique of Gomori were used.<sup>cf. 8</sup> Since the total number of mice examined was in the hundreds and the changes observed were fairly similar, it was considered proper to describe them without giving the numbers of specimens studied except when the number was small. Large numbers of mice have been preserved *in toto* and are available to those interested.

*Nomenclature.* As *dependent* or conditioned tumors will be designated those requiring for their growth destruction of the host's thyroid gland (absence of thyroid hormone); as *autonomous* tumors, those which grow in normal hosts. Dependency or autonomy are relative and quantitative terms.<sup>6</sup> By *late autonomy* is meant the development of small tumors in normal mice injected with dependent strains of tumors, after a very long (over 8 months) latency period; when transplanted, these tumors grew readily in normal hosts. Destruction of the thyroid by  $I^{131}$  is termed *radiothyroidectomy*. The term "gonad-stimulating hormone" will be used because a separation into follicle stimulating and luteinizing hormonal activities was not carried out. The histologic changes indicate that stimulation by these two hormones occurred concurrently, with the activity of one or the other in excess.

#### *Pituitary Tumors*

Halmi<sup>8</sup> traced the histogenesis of the pituitary tumors to beta cells even though they are chromophobic and give neither the periodic acid-Schiff reaction for 1, 2 glycols nor the aldehyde-fuchsin reaction of Gomori. Sudden loss of weight, slight bulging of the cranium over the occipital region, listlessness, ruffled fur (Fig. 1), and uncoordinated motions marked the terminal stage, when the animals either died suddenly or were sacrificed. At death the primary tumors (Fig. 2) measured 6 to 12 mm. across. The microscopic appearance of primary tumors is described and illustrated by Halmi.<sup>8</sup> Although some of the primary tumors exhibited a fair degree of anaplasia and many were locally invasive, none of 11 successfully grafted on thyroidectomized mice grew in normal animals.



The first generation grafted tumors (Figs. 3 to 7) had many features in common with the primary growth. The predominant cells were chromophobe and free of granules. The amount of cytoplasm was usually moderate. The clusters of very large cells of thyroidectomy type, often present in the first grafts (Fig. 3), soon vanished in the course of subpassages, the cytoplasm of well established tumors being scant or moderate in amount. Features of anaplasia were absent. The tumor cells usually formed solid masses with no definite pattern (Fig. 7). Rarely tubular formations (Fig. 5) were noted, with empty or sometimes blood-filled lumina lined more frequently by tumor cells than by endothelial cells. The large periodic acid-Schiff-positive intranuclear inclusions characteristic of primary tumors<sup>8</sup> were also frequently noted in grafted dependent tumors (Fig. 4). The stroma of the dependent tumors was invariably scant and correspondingly the tumors were soft, almost pulpy. Retrogressive changes occurred, as with other grafted tumors, when the tumors were large. Terminally, the bulk of the tumor often underwent necrosis. The grafted tumors weighed 4 to 8 gm. at death.

Retrogressive changes with fibrosis were much more marked with *autonomous* tumors which could usually be identified on gross inspection by their firmness and variegated appearance. The latter is due to scattered areas of necrosis, congestion, hemosiderosis, and fibrosis (Figs. 16 to 19). The greatest degree of retrogressive change occurred in late autonomous tumors which grew very slowly over long periods. While the dependent tumors were composed of masses lacking histologic criteria of malignancy, marked anaplasia developed in the course of transplantation as the tumors gained autonomy. A cytologic correlation of dependency and autonomy is yet to be made. Cells of autonomous tumors varied greatly in size and shape; cells with micro-nuclei and macro-nuclei and giant cells were common (Figs. 17 to 19). Nuclear chromatophilia increased and nuclear-cytoplasmic ratio decreased. These features of malignancy were acquired gradually. It has not been possible to state on the basis of microscopic appearances whether or not a tumor has already acquired autonomy. After several subpassages, however, the microscopic appearance of the autonomous tumors invariably indicated a high degree of malignancy. A study of frequency and character of mitotic figures in relation to tumor type remains to be made.

Metastases in the regional lymph nodes were common with both dependent (Fig. 8) and autonomous growths (Figs. 13 to 15), but they were larger with the latter and more widespread, extending

throughout the para-aortic abdominal chain of lymph nodes as far as the diaphragm (Fig. 14). The microscopic appearance of the metastases resembled that of the primary growth. In several mice there were extensive bilateral metastases in the ovaries (Fig. 15) and, in a few there were metastases also in the liver. A yellow-brown pigment present in large mononuclear cells of the tumor and in draining lymph nodes gave an iron reaction.

In one experiment transplantations were made with an iliac lymph node replaced by metastasis from a dependent tumor. The grafts took in radiothyroidectomized hosts but not in normal hosts, indicating that these metastatic tumors remained dependent.

In one experiment that will not be detailed, intrasplenic grafts were made in order to find out if the liver metabolizes the pituitary hormones of these grafts as it does those of gonadal grafts. In 2 of 4 mice grafted in the spleen there were extensive metastases in the liver (Figs. 9 and 11) and both animals developed a malignant lymphoma (Figs. 9, 10, and 12). The latter disease is very rare in this strain. The induction of lymphoma by growth hormone has been reported by Moon *et al.*<sup>9</sup> and following pituitary grafts by Silberberg and Silberberg<sup>10</sup> who attributed the leukemogenic effect to ACTH. The relation of anterior pituitary activity to tumor induction has been reviewed by Lipschutz.<sup>11</sup>

#### *Thyroid Gland*

The changes in the thyroid gland caused by radiothyroidectomy have been amply described by Gorbman,<sup>12</sup> Goldberg *et al.*,<sup>4,13,14</sup> Rugh,<sup>15,16</sup> and Maloof *et al.*<sup>17</sup> The following are observations on mice bearing grafted pituitary tumors with some supplementary findings on the effects of radiothyroidectomy.

*Normal Hosts.* Thyroid stimulation was apparent in all normal mice bearing autonomous tumors, the degree of enlargement and of adenoma formation being in direct relation with the length of the tumor-bearing period. In normal mice in which the tumors gained autonomy many months after the graft was made (late autonomy), the thyroid glands were tremendously enlarged (Figs. 34 and 35), the weight of this organ increasing from approximately 0.8 mg. to over 6 mg. even when the grafted tumors measured but a few millimeters across. In numerous mice the grafts were not identified but were suspected because of the greatly enlarged thyroid glands, and subsequently minute growths were located after some gross or microscopic search. The tumor nodules so identified measured but 1 to 2 mm. in diameter in many mice in which thyroid enlargement gave evidence of excessive

secretion of thyrotrophin by grafted pituitary tumors (Figs. 24 and 25). Whether these delayed minute growths represent the acquisition of late autonomy or failure of a normal host to restore its homeostatic balance in the presence of grafted, dependent pituitary tumors remains to be studied. If the cells were autonomous, explanation is needed why they remained localized at the site of injection; and if not normal, homeostasis would call for their depression.

Soon after establishment of autonomy the tumors gained in proliferative vigor and killed the mice a few months after grafting, when the tumors weighed 2 to 4 gm. The thyroid glands of these mice were several times the normal size but were not as large as those with late autonomous tumors; in sections they invariably gave evidence of marked thyrotrophic stimulation (Figs. 24 and 25). In the course of successive passages the thyroid stimulation was less pronounced. This is due in part to a decrease in the tumor-bearing period and in part to a diminution of thyrotrophin secretion by the tumors, as indicated by bioassays of tumors and of blood of tumor-bearing animals.

Microscopic examinations (Figs. 24, 25, and 36) have shown the well known evidences of stimulation by thyrotrophin, namely, resorption of colloid, enlargement of the epithelial cells, cytoplasmic colloid masses, vacuolization of the cells, and, after sustained stimulation, formation of increasing numbers of adenomas, some of which were papillary (Figs. 36 to 38, *cf.* normals: Figs. 20 and 21); colloid formation became diminished. Radioautographs have indicated a fairly uniform uptake in different follicles of the normal gland, a great variability in stimulated follicles, and almost complete lack of uptake in the adenomas.<sup>6</sup>

In mice with greatly enlarged thyroid glands the neck was markedly swollen over the gland, and frequently there was a brownish discoloration of hair (normally brown-black) in this region.

*Radiothyroidectomized Hosts.* In almost all mice receiving approximately 300  $\mu$ c. of  $I^{131}$  the thyroid glands were absent and the characteristic chronic radiation damage, such as stenosing arteritis, hemosiderosis, and fibrosis, marked the site of the gland (Figs. 28, 32, and 33). Stenosing tracheitis, also noted by Silberberg *et al.*,<sup>18</sup> was occasionally encountered (Fig. 29). In animals receiving smaller doses (75 to 200  $\mu$ c.) and in some receiving as much as 300  $\mu$ c. some thyroid tissue was present (Figs. 22 and 23), but this was evidently incapable of being stimulated by thyrotrophin or undergoing compensatory hyperplasia, even though the blood of these animals contained large amounts of thyrotrophin.<sup>19</sup> Many of these thyroid cells often

failed to form acini and in those which did the lumina were devoid of, or poor in, colloid. The thyroid cells varied greatly in size and shape; the nuclei of many were hyperchromatic; the cytoplasmic-nuclear ratio was reduced and occasional cells had the morphologic features of carcinoma cells (Figs. 22 and 23). The presence of such cells in heavily irradiated fields is well known (*cf.* Maloof *et al.*<sup>17</sup>); their biologic potentialities have, however, not been analyzed, so far as I know. They appear soon after irradiation, remain *in situ*, and are not known to proliferate as cancers. Failure to respond fully to thyrotrophin may explain why thyroid glands partially destroyed by  $I^{131}$  do not inhibit the growth of dependent tumors.<sup>6</sup> The thyroid glands of mice receiving partially destructive doses of  $I^{131}$  exhibited cytologic evidence of stimulation, but proliferation to the extent of restoration to normal was absent. Rarely pituitary tumors coexisted with thyroid adenomas exhibiting morphologic evidence of stimulation by thyrotrophin (Fig. 26).

*Metastases.* Invasion of blood vessel by thyroid tissue (Fig. 39) and metastasis of thyroid "adenomas" in regional lymph nodes were rare findings, but no special search was made to detect the spread of thyroid adenomas. Pulmonary metastases of the thyroid adenomas (as occurring in thiouracil-treated animals) were not observed. The possibility of neoplastic transformation was tested by grafting these adenomas in muscle of mice bearing autonomous pituitary tumors or by injecting minute thyroid particles intravenously. Thus disseminated "tumor" nodules were produced in the lungs and these exhibited the same degree of stimulation as the animal's own thyroid gland (Figs. 40 and 41) but did not undergo malignant transformation. In control normal mice bearing grafted thyroid fragments, the latter could not be identified at necropsy. Four normal mice that had been given intravenous injections of thyroid cells and intramuscular grafts of autonomous tumors died suddenly when the grafted pituitary tumors measured but 1 to 2 cm. across. All had minute disseminated nodules of thyroid tissue in the lungs (Figs. 42 to 45), extensive central necrosis of the liver (Fig. 46), and thrombosis of the cardiac auricles. That these animals died of thyrotoxicosis was suggested by the sudden occurrence of death with heart failure and hepatic necrosis, with no anatomical change indicative of another mode of death. Further work is required to find out whether thyrotoxicosis was caused by the rapid discharge of thyroid hormone or by its heightened production from these pulmonary thyroid nodules and to establish if the lung might serve as an inactivator of excessive quantities of thyroid hormone.

*Changes in the Thyroid Region by  $I^{131}$  Treatment.* Hyperplasia in the tracheal epithelium, notably in juxtaposition to the irradiated thyroid gland, was seen only in the presence of inflammation, and it never proceeded to tumor development. On the contrary, the mucosa, submucosa, and cartilage of the larynx, notably the parts in juxtaposition to the thyroid gland, often appeared injured (*cf.* Figs. 27 to 29). There was atrophy of epithelium, replacement of ciliary epithelium by squamous cells, atrophy of mucous glands of the submucosa, chronic inflammation, degenerative changes in the cartilage with calcification (Fig. 28), but never changes known to be precancerous or cancer in any of these structures.

The only neoplasm observed about the thyroid gland occurred in an overlying submaxillary gland. The tumor measured 15 by 15 by 12 mm. across, involved symmetrically both lobes of the submaxillary gland, and did not invade the thyroid gland or skin. The microscopic appearance indicated an anaplastic growth, probably a carcinoma, with osteogenesis in the stroma (Figs. 68 and 69), or an extrasosseous osteogenic sarcoma. Osteogenesis is a well known feature of salivary tumors. In view of this finding the morphologic changes in the submaxillary gland of radiothyroidectomized mice deserve special study. This organ, overlying the thyroid gland, must receive large quantities of gamma irradiation in the course of radiothyroidectomy.

A systematic study of the *parathyroid gland* was not made. In general, this organ, when identified in radiothyroidectomized mice, was smaller than normal and contained an excessive amount of connective tissue, notably in parts nearest to the thyroid gland (Figs. 30 and 31). The parathyroid damage was attributed by Rugh<sup>15,16</sup> to a diminished blood supply. In addition, local beta irradiation damage is indicated by the characteristic appearance of the lesion, that is, localization of fibrosis to parts in juxtaposition to the thyroid gland. Lack of morphologic evidence of compensatory hyperplasia is noteworthy.

#### *Gonads and Accessory Sex Organs*

*Ovary.* In radiothyroidectomized animals with *primary pituitary tumors* the ovaries were invariably small and yellow, due to overgrowth of luteinized cells, and ova were absent (Fig. 52). Occasional nodular areas of lutein cells (Fig. 53) suggested the beginning of a luteoma. Atrophy of the female gonads in mice with primary pituitary tumors, as noted by Gorbman,<sup>12</sup> is the rule but with small tumors and "pretumors" some ovarian stimulation was evident. In contrast to the ovaries of x-rayed mice, to which this picture bears close resemblance,



tubular down-growth of the germinal epithelium and formation of expanding nodules of granulosa cells were invariably absent. It is possible that the ovaries of these 13 to 16 months' old animals were non-responsive to gonadotrophins or, more likely, that the primary thyrotrophin-secreting pituitary tumors depressed or destroyed the gonadotrophin-secreting cells. Assessment of gonadotrophins of these pituitary tumors and of the blood of such tumor-bearing mice, and the study of the morphology and responsiveness of ovaries in the course of pituitary tumorigenesis by I<sup>131</sup> may clarify this problem. The studies and assays of Anderson and Bates<sup>20</sup> suggest the presence of minute amounts of gonadotrophins in dependent tumors.

A remarkable difference was noted between radiothyroidectomized and normal mice bearing grafted pituitary tumors. The ovaries of *radiothyroidectomized* mice bearing large grafted tumors were greatly enlarged, measuring as much as 5 to 6 mm. across, and were studded with hemorrhagic and cystic follicles similar to those given by gonadotrophins, which characterize the pregnancy reaction of Aschheim-Zondek (Figs. 54 to 57). In several mice spontaneous rupture of a hemorrhagic follicle caused exsanguination into the peritoneal cavity. The sequence of changes appears to be as follows: hastened maturation of follicles, accumulation of liquor, hemorrhage in follicles, and luteinization of stromal cells. The relative degree of stimulation by follicle-stimulating hormone and luteinizing hormone, respectively, varied with different animals. Correlation of morphologic appearances with bioassays will be required to explain this variability. Luteinization of the stromal cells was predominant when the follicles were few or altogether absent ("burned out"). In some mice with slowly growing tumors the ovaries were yellow and of approximately normal size or even smaller than normal.

With *autonomous tumors* in normal hosts the gonads and accessory sex organs were either normal or, less often, smaller than normal, but never distinctly stimulated. Autonomous tumors carried in radiothyroidectomized mice did cause gonadal stimulation but this was slight and infrequent. To investigate this problem further, 8 animals bearing large grafted tumors were killed, all having received grafts of the same autonomous tumor on the same day, 4 recipients being radiothyroidectomized and 4 normal. All radiothyroidectomized mice exhibited a marked ovarian and uterine stimulation while in normal mice these organs were atrophic. This finding has since been amply confirmed in the course of routine necropsies of large numbers of mice, supporting the assumption that lack of thyroid hormone enhances sensitivity to



gonadotrophins. It is known (Bischoff *et al.*<sup>21</sup>) that thyroidectomy will increase the ovarian response to injections of hypophyseal gonadotrophins. Thyroxin is able to counteract the influence of thyroidectomy. Bischoff *et al.* have explained this by a decrease in the rate of exchange of the body fluids brought about by thyroidectomy, but reduced metabolism rate goes with conservation of gonadotrophin. Thyroidectomy alone (without grafted tumors) did not cause ovarian enlargement.

The *uteri* of mice bearing pituitary tumors in radiothyroidectomized hosts present one or a combination of the following changes: thickening of the uterine wall without dilatation and elongation (Fig. 54) due to predominantly stromal and muscular hyperplasia, or a marked elongation and dilatation. The former change was noted with transplantable granulosa cell tumors and was attributed to secretion of granulosa cells (estrogens); the latter was noted with luteomas and was attributed to secretion of lutein cells (progestins). The usual change with dependent tumors is that of hyperplasia with dilatation and elongation of the uterine horns indicative of a mixed type of stimulation. Less frequently the uterine horns exhibit a tremendous cystic dilatation as shown in Figure 55. This puzzling alteration has not been noted in hosts carrying granulosa tumors, luteomas, or Leydig cell tumors. The uterine changes, obviously secondary to ovarian stimulation and accordingly more marked with dependent than with autonomous tumors, are absent in normal hosts. Incidentally, this suggests the use of radiothyroidectomized animals in assays for gonadotrophins, including those of pregnancy.

*Testes and Accessory Male Organs.* In radiothyroidectomized mice bearing dependent tumors there is a marked hyperplasia and hypertrophy of the Leydig cells (Fig. 64). This change is analogous to the ovarian stimulation which has been discussed, and is not found in normal mice bearing autonomous tumors. In mice bearing dependent tumors (and having stimulated Leydig cells) the seminal vesicles are greatly enlarged and filled with secretions. In normal mice with autonomous tumors the seminal vesicles are smaller than normal. In mice with dependent tumors the sex features of the submaxillary glands are exaggerated in both sexes. The prostates of the males are likewise large. These organs have not been adequately studied.

*Mammary Gland.* In female mice bearing primary pituitary tumors and having atrophic luteinized ovaries the mammary ducts are greatly hypertrophied and hyperplastic (Figs. 65 to 67). Rarely the ducts are distended with some milky fluid. This change is noted also in females bearing slowly growing, grafted, dependent pituitary tumors and hav-

ing small lutein cell-laden ovaries, but not in males. Bates<sup>20</sup> failed to find lactogenic hormone in dependent pituitary tumors, and the normal cells of pituitary bodies in which this alteration is usually encountered are likely to be replaced by tumor cells. I am at a loss to explain the pathogenesis of this change. The mammary glands in the mice with greatly stimulated ovaries are not enlarged.

*Hyperplasia and Ectasia of the Extrahepatic Biliary Ducts*

A common finding in mice bearing large dependent tumors<sup>22</sup> is hyperplasia of the epithelium of the extrahepatic ducts, most marked at the ampulla of Vater, with hyperplasia of the submucosa and cystic dilatation of the anatomically patent ducts (Fig. 48).

The following confirmatory and supplementary information is based on newer observations. Rupture of the cyst, with large amounts of viscous, slightly bile-stained fluid in the peritoneal cavity, was a common terminal event; yet evidence of biliary tract obstruction was absent when tested post mortem by injection of fluid into the cysts. Figure 51 is a cross section at the ampulla of Vater; the arrow points to the orifice of the duct. Often fibrous adhesions were formed between the large cyst, the anterior abdominal wall, and loops of intestine adjacent to the cyst. The cystic duct was often thickened, the hepatic duct occasionally, but the gallbladder usually appeared normal; rarely it was somewhat dilated. Hyperplasia of the intrahepatic duct, if present at all, was localized to the region in immediate continuity with the extrahepatic duct (Figs. 49 and 50). Hyperplasia appears to precede dilatation. The changes were most marked in the region adjacent to the junction of hepatic, cystic, and common ducts or at the ampulla, with or without a macroscopic change at other sites. Obstructive jaundice was absent in spite of the tremendous dilatation of the extrahepatic biliary tract and, while acute inflammation of the head of the pancreas in the region of the ampulla was common, intrahepatic cholangitis was but rarely noted.

Cystic dilatation of the extrahepatic biliary tract occurred in most radiothyroidectomized mice bearing large, dependent, pituitary tumors. It was usually associated with evidence of gonadal stimulation and was seen but once without it, while gonadal stimulation without changes in extrahepatic biliary tract was frequent (Table I). Thus the two alterations appear to be caused by different and probably hormonal mechanisms. Dilatation of the extrahepatic biliary tract was not seen in mice bearing grafted hormone-secreting granulosa cell tumors, luteomas, or Leydig cell tumors. Thus the hosts exhibiting this change

are characterized by a lack of thyroid hormone, by hypersecretion of thyrotrophin, and usually by excessive stimulation by gonadotrophins; but how these factors act to bring about the biliary duct ectasia and whether other factors are involved are unknown. The report of Gardner *et al.*,<sup>23</sup> who noted a similar change in mice receiving estrogens

TABLE I  
Relation of Tumor Size to Gonadal Stimulation and Cystic Dilatation of the Extrahepatic Biliary Tracts

No. of strain	Tumor dependent (D) or autonomous (A)	Host	Tumor size	Number of mice			
				Gonad+* Duct+	Gonad+ Duct-	Gonad- Duct+	Gonad- Duct-
77	D	Athyroid	++				9
			+++	7			
6	D	Athyroid	++				4
			+++				13
101	D	Athyroid	++		8		2
			+++	2	25		5
124	D	Athyroid	++	5			
			+++	13			
3	D	Athyroid	++	18	2		
			+++	7			
19	D	Athyroid	++	20	1		1
			+++	20	1		
163	D	Athyroid	++	25	1	1	4
Total	D	Athyroid	++	68	12	1	20
			+++	49	26		18
			++ and +++	117	38	1	38
19	Late A	Normal	+				13
163	Late A	Normal	++		5		
3	Late A	Normal	++				4
3	Late A	Normal	+++				5
Total	Late A	Normal	+ to +++		5		22
3	A	Athyroid	++		8		7
			+++		42		25
3	A	Normal	++		3		12
			+++				74

\* Evidences of gonadal stimulation present (+) or absent (-).

Hyperplasia and dilatation of the extrahepatic biliary ducts present (+) or absent (-).

over long periods of time, is no less puzzling. In my experiments biliary duct ectasia occurs in mice of both sexes and may be present in mice with grafted tumors subjected to gonadectomy a few weeks or months before death.<sup>22</sup>

#### Adrenal Gland

Changes indicative of overproduction of ACTH have been consistently absent in mice bearing either dependent or autonomous tumors or in those used for bioassays of tumor extracts. Adrenal

glands of radiothyroidectomized mice with dependent tumors were normal or smaller than normal. A characteristic change noted consistently in the reticular zone was replacement by large cells with pyknotic nuclei and bulky "foamy" sudanophilic cytoplasm (Figs. 60 and 61). These degenerative changes in the reticular zone are similar to the "brown" degeneration seen by Cramer and Horning<sup>24</sup> in animals after prolonged administration of estrogens. Some of the yellow material in the "foam" cells was also acid-fast. Studies with transplantable luteomas suggest that the yellow material in degenerating lutein cells is probably derived from a steroid hormone of these cells. "Healthy" secreting lutein cells are not acid-fast and are weakly, if at all, sudanophilic, and acid-fast granules appear in the lutein cells in areas of degeneration.

In normal mice with autonomous tumors the adrenal glands were enlarged by excessive fatty deposit in cells of the reticular zone (Figs. 62 and 63) and not by hyperplasia. The beginning of this change is shown in Figure 63. Large fat globules accumulate in the cytoplasm of the cells and push the nucleus to the periphery, thus creating a "signet ring" cell. Such changes were consistently absent in normal control mice and in those bearing either feminizing granulosa cell tumors or masculinizing luteomas or Leydig cell tumors. The fascicular and glomerular zones showed no conspicuous changes. A special histochemical study of the adrenal glands remains to be undertaken.

### *Thymus*

The thymus was characteristically atrophic in radiothyroidectomized mice or in those bearing grafted dependent tumors or large primary tumors, but in normal mice bearing autonomous tumors and in those with small primary tumors it was frequently normal. If the latter is taken as supporting evidence for the assumption that the autonomous tumors do not secrete ACTH, an explanation is needed for the characteristic atrophy of the thymus in radiothyroidectomized mice bearing dependent tumors. Atrophy (accidental involution) of the thymus is a usual event in mice bearing large transplanted tumors, and a "chronic stress" with non-specific hypersecretion of the adrenal cortex is the usual explanation. Radiothyroidectomized mice lose much weight, while normal tumor-bearing mice are well nourished. Radiothyroidectomized mice are hypothyroid, normal tumor-bearing mice are hyperthyroid, and the latter state is known to be associated with lymphoid hyperplasia. Persistence of the thymus with autonomous

tumors is therefore the more meaningful finding indicating lack of hypersecretion of the adrenal gland.

### *Pancreas*

In normal mice bearing autonomous tumors the pancreas was gray-pink and conspicuously enlarged. Microscopic examinations disclosed, in addition to hyperplasia, the fusiform cleft-like spaces in exocrine cells illustrated in Figure 47. The character of this change, its frequency and meaning, require special studies.

### *Increase in Blood Volume in Normal Mice Bearing Autonomous Tumors*

The increase in blood volume in normal mice bearing autonomous tumors was indicated by the unusually large amount of blood (1.5 to 2 ml.) obtained for assays by cardiac puncture. Blood volume determinations were not made, but the amount obtained from normal hosts in the same transplantation series was almost double that from radiothyroidectomized hosts. Absence of the characteristic morphologic features of hypervolemia<sup>25</sup> indicates that the blood volume increase was only moderate as compared to that in mice with granulosa tumors.

### *Survey of Frequency and Constancy of Secondary Changes*

Table II surveys the salient secondary changes observed in different hosts. The presence or absence of gonadal stimulation and formation of cystic dilatation of the extrahepatic biliary tracts is tabulated in Table I on the basis of a random sampling of 392 mice.

Dilatation of biliary ducts occurred only in radiothyroidectomized hosts and was always accompanied by gonadal stimulation with a single exception. This mouse, preserved *in toto*, was carefully re-examined. It was found to have atrophic gonads and uterus and an enormously distended common duct. The reverse, gonadal stimulation in the absence of biliary duct ectasia, was encountered frequently.

The following strain differences are suggested by Table I: Tendency to give rise to autonomous cells (strains 19, 163, and 3) or the lack thereof (strains 77, 6, 101, and 124); marked gonadal stimulation with very large tumors, in absence of biliary tract ectasia (strain 101); almost invariable presence of both ductal ectasia and gonadal stimulation with large tumors (several strains), or the lack of both (strain 6); consistently slow growth rate during a period of 2½ years (strains 6 and 77) or steadily gaining proliferative vigor (strain 3). Whether

these differences, noted at the conclusion of these studies, are significant and can be correlated with morphologic and cytologic features of the pituitary tumors and of the pituitary bodies of their hosts remains to be studied.

Thyroid stimulation was apparent in all normal mice bearing tumors, without distinct changes in the gonads and extrahepatic biliary tract. Several normal mice grafted with autonomous pituitary tumors, in which the tumor-take was not identified on gross examination, exhibited evidence of thyroid stimulation. Gonadal changes were conspicu-

TABLE II  
*Changes Secondary to  $I^{131}$  Induced Pituitary Tumors in Different Hosts*

	Thyroid gland	Ovaries, uteri	Biliary tract ectasia	Adrenal glands	Thymus
Primary tumor	Absent	o	o	o	Normal or atrophic
Dependent tumor Host $I^{131}$ treated	Absent	o to +++	o to +++	Degenerative changes in reticularis	Atrophic
Autonomous tumor Host normal	+ to +++*	o	o (to +)	Degenerative changes	Normal or atrophic
Host $I^{131}$ treated	Absent	o to ++	o to ++	Degenerative changes	Atrophic

\* Plus signs indicate degree of specific hormonal stimulation.

ous at necropsy in most radiothyroidectomized mice bearing dependent tumors of medium or large size. They were less often present and were much less marked in mice with autonomous tumors in radiothyroidectomized hosts, and lack of gonadal stimulation or gonadal atrophy were the usual findings with autonomous tumors in normal hosts.

Changes in the extrahepatic biliary tract occurred only in mice bearing large tumors and were often absent in mice with tumors of medium size and exhibiting gonadal changes (Tables I and II).

#### *Quantitative Assays for Thyrotrophin in Different Types of Tumors*

The quantity of thyroid-stimulating hormone in tumors and in the blood of tumor-bearing hosts, assayed in numerous experiments in chicks and mice, will be fully reported later.<sup>19</sup> In each experiment three parameters were recorded: morphologic stimulation of the thyroid glands examined as unknowns, thyroidal retention of a tracer dose of  $I^{131}$ , and total body retention of  $I^{131}$ . The results indicate that dependent tumors contain about as much thyrotrophin as primary



tumors and the latter several times as much as the normal pituitary body calculated on the basis of unit weight. Because of the uncertainty of identifying thyrotrophin-secreting cells in the normal pituitary body, no calculation can be made on the basis of the number of such cells. Autonomous tumors contain much less hormone than dependent tumors. Similarly the blood of mice with dependent tumors contains large quantities of thyrotrophin and that of mice with autonomous tumors contains much less.

#### DISCUSSION

*Dependency and Autonomy.* Dependent tumors are those in which apparently normal cells proliferate in an altered host; autonomous tumors are those in which permanently altered cells proliferate in normal hosts.<sup>26</sup> All of 11 primary tumors bioassayed proved dependent, even though they regularly metastasized to regional lymph nodes in athyroid hosts. Such lymph node metastases assayed were found to be as dependent as the primary tumors. The thyroid adenomas occurring in normal hosts bearing thyrotrophin-secreting autonomous pituitary grafts and in hosts treated with thiouracil are likewise dependent tumors even though they can metastasize to regional lymph nodes or lungs. Thyroid tumors induced by thiouracil have the ability to metastasize to the lungs and yet are dependent on interference with thyroid hormonal synthesis.<sup>cf. 26</sup> Their driving force is thyrotrophin excess. Successive transplantation of conditioned thyroid tumors by Morris *et al.*<sup>27</sup> led to autonomous thyroid tumors, as successive transplantation of our dependent pituitary tumors led to the development of autonomous pituitary growths. It is being debated whether dependent pituitary or thyroid growths should be called true neoplasms. A discussion of the terminology of "neoplasia" and "cancer" is, at present, fruitless; what is wanted is better knowledge of the alterations in the hosts and in the cells, which accompany formation of dependent and autonomous growths.<sup>cf. 26</sup>

*Identity of the Thyrotrophin-Secreting Cells.* The essential identity of all seven pituitary tumors induced by radiothyroidectomy and transplanted in series deserves special emphasis. All are chromophobic and possess similar cytologic features, behave similarly in the course of serial transplantations, and cause the same secondary changes. All produce thyrotrophin in large quantities in radiothyroidectomized hosts. Subtle differences among the various strains may exist, but the essential identity of the seven strains studied deserves emphasis.

According to current concepts, reviewed by Halmi,<sup>8</sup> the thyrotropes are beta cells characterized by giving a positive periodic acid-Schiff's



and aldehyde-fuchsin reaction. The tumor cells here described are doubtless thyrotropes and, although they are probably derived from beta cells,<sup>8</sup> they give neither of the above reactions. The explanation that in radiothyroidectomized hosts the tumor cells discharge thyrotrophin almost as fast as they produce it, carries with it some uncertainty. With autonomous tumors secreting thyrotrophin and growing in normal hosts, the thyroid hormone produced should retard release of thyrotrophin and therefore give the periodic acid-Schiff reaction. The present studies indicate that current criteria are inadequate to identify a thyrotrophin-secreting cell.

*Experimental Pituitary Tumors of Different Types.* Adenomas of the pituitary body have been described in gonadectomized mice.<sup>28</sup> Control data are wanting on the spontaneous incidence of these tumors and on the types of hormones, if any, they secrete. Compensation by cells of the adrenal cortex might prevent complete absence of estrogens and progestins, but complete absence of gonadal hormones could be achieved by combined removal of gonads and adrenal glands and maintaining life with cortisone. Dunning *et al.*,<sup>29</sup> confirming others, induced pituitary tumors by sustained treatment of rats with stilbestrol, and have shown by transplantation tests that these tumors are conditioned neoplasms, but they did not study their secretions. Monomorphous transplantable ACTH-secreting pituitary tumors have been isolated recently.<sup>30</sup> Thus, if the estrogen-induced tumors secrete gonadotrophin, at least three types of pituitary tumors can be made available for controlled investigations for further research on cell type and function. The question is whether the conditioned tumors are composed of essentially normal cells and whether the chromophobes are reserve cells; if so, it may be possible to bring about a change in the tumor cell types in hypophysectomized mice by creating a need for one hormone while substituting for all others.

*Retrogressive Changes in Autonomous Tumors.*<sup>cf. 26</sup> The pathogenesis of retrogressive changes is complex, and its better understanding calls for special studies. The rôle of deficient vascular supply in causing retrogressive changes is well documented. Greene<sup>31</sup> has shown that such changes may be due to immunity reactions and it is conceivable that, as the tumor becomes autonomous, an antigenic change accompanies a genic change. Furthermore, the remote possibility that thyroid hormone or related substance may exert some effect on thyrotrophin-secreting cells has to be considered. If the latter factor is operative, the same tumor strain is expected to behave differently in normal and radiothyroidectomized hosts. The maximum degree of

retrogressive changes with fibrosis was noted with highly secreting, slowly growing, late autonomous tumors. In rapidly growing autonomous tumors retrogressive changes, notably fibrosis, were less marked.

*Further Unsolved Problems.* Formation of adenomas in the thyroid glands of mice with autonomous tumors and their spread to regional lymph nodes deserve emphasis. Malignant transformation of thyroid adenomas could probably be achieved by successive grafts of thyroid adenomas in mice bearing autonomous thyrotrophin-secreting tumors. Since such a transformation of thiouracil-induced adenomas to carcinomas has already been demonstrated by Morris *et al.*,<sup>27</sup> this problem was not pursued further.

Secretion of some gonadotrophins by the tumor cells in radiothyroidectomized hosts probably occurs.<sup>20</sup> The observations made indicate that several factors might determine the presence or absence of gonadal stimulation in mice with pituitary tumors: (a) gonadotrophin secretion by the tumor cells, (b) enhancement of sensitivity of the gonads to gonadotrophins in the absence of thyroid hormone, (c) influence on gonadotrophin secretion by the normal pituitary cells, (d) altered responsiveness of gonads (*e.g.*, I<sup>131</sup> injured or aged) to gonadotrophins.

The hyperplasia of the pancreas and the formation of cleft-like spaces in acinar cells in normal hosts bearing autonomous tumors remain to be elucidated. Laqueur<sup>32</sup> is of the opinion that this is related to functional activity of the cells and may represent negative images of unstained mitochondria. He noted that the clefts were more prominent when the zymogen granules were less densely packed or even absent and when the basophilic substance was prominent.

One lesson learned from the study of transplanted hormone-secreting tumors is that changes in the host in which the tumor originates seldom disclose the hormone-secreting potentialities of the tumor cells. This is true for estrogen, thyrotrophin, and ACTH-secreting tumors studied by me, injury to the target organ being the main inciting factor of these tumors. When the primary pituitary tumors are large, compression atrophy of normal elements causes a secondary atrophy of all organs stimulated by the pituitary body. The hormonal potencies of "spontaneous" or primary pituitary tumors are better analyzed in hosts bearing small tumors which have not destroyed this organ completely.

#### SUMMARY AND CONCLUSIONS

Morphologic characteristics of dependent and autonomous transplanted pituitary tumors, originally induced by radiothyroidectomy, and the secondary changes caused by these tumors are described. De-

pendent tumors are composed of chromophobe cells fairly uniform in size and shape; they lack the features of cancer cells. Autonomous tumors exhibit features of anaplasia characteristic of a malignant neoplasm. Retrogressive changes (necrosis, fibrosis, hemorrhage, and hemosiderosis) are more often encountered and are more marked in autonomous growths. Factors to explain the latter are discussed.

Chromophobe cells are capable of secreting large quantities of thyrotrophin as indicated by assays of the tumors and of blood of tumor-bearing animals.

Secretion of thyrotrophin by these tumors occurs invariably, more by dependent than by autonomous growths. In autonomous tumor-bearing normal hosts secretion of thyrotrophin causes extensive stimulation of the thyroid gland of the host with formation of adenomata which may invade the blood vessels and spread to regional lymph nodes.

Gonadal stimulation is common in radiothyroidectomized hosts bearing large tumors but is absent in normal hosts bearing autonomous tumors. This may be due to secretion of minute quantities of gonadotrophin by some of the tumor strains studied and to some other factors which are discussed.

The morphologic changes in hosts bearing these tumors suggest lack of secretion of pituitary hormones other than thyrotrophin, with the possible occasional exception of traces of gonadotrophin.

Cystic dilatation of the biliary tract was common in radiothyroidectomized mice, notably in the presence of gonadal stimulation.

The following alterations seen in mice bearing pituitary tumors remain to be explained: retrogressive changes in the reticular zone of the adrenal gland; hyperplasia with cystic dilatation of the extrahepatic biliary tracts; extensive ductal hyperplasia of the mammary gland of female radiothyroidectomized mice; cleft-like spaces in pancreatic cells; pathogenesis of sudden death with signs suggestive of thyrotoxicosis in mice bearing intrapulmonary thyroid grafts.

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#### REFERENCES

1. Gorbman, A. Tumorous growths in the pituitary and trachea following radio-toxic dosages of  $I^{131}$ . *Proc. Soc. Exper. Biol. & Med.*, 1949, 71, 237-240.
2. Furth, J., and Burnett, W. T., Jr. Hormone-secreting transplantable neoplasms of the pituitary induced by  $I^{131}$ . *Proc. Soc. Exper. Biol. & Med.*, 1951, 78, 222-224.

3. Furth, J., Gadsden, E. L., and Burnett, W. T., Jr. Autonomous transplantable pituitary tumors arising in growths dependent on absence of the thyroid gland. *Proc. Soc. Exper. Biol. & Med.*, 1952, 80, 4-7.
4. Goldberg, R. C., and Chaikoff, I. L. On the nature of the hypertrophied pituitary gland induced in the mouse by  $I^{131}$  injections, and the mechanism of its development. *Endocrinology*, 1951, 48, 1-5.
5. Gadsden, E. L., and Furth, J. Effect of thyroid hormone on growth of thyrotrophin-secreting pituitary tumors. *Proc. Soc. Exper. Biol. & Med.*, 1953, 83, 511-514.
6. Furth, J., Burnett, W. T., Jr., and Gadsden, E. L. Quantitative relationship between thyroid function and growth of pituitary tumors secreting TSH. *Cancer Research*, 1953, 13, 298-307, and unpublished data.
7. Gude, W. D. Modified Martins-Mallory stain for mouse pituitary gland. *Stain Technol.*, 1953, 28, 161-162.
8. Halmi, N. S., and Gude, W. D. The morphogenesis of pituitary tumors induced by radiothyroidectomy in the mouse and the effects of their transplantation on the pituitary body of the host. *Am. J. Path.*, 1954, 30, 403-419.
9. Moon, H. D., Simpson, M. E., Li, C. H., and Evans, H. M. Neoplasms in rats treated with pituitary growth hormone. I. Pulmonary and lymphatic tissues. *Cancer Research*, 1950, 10, 297-308.
10. Silberberg, M., and Silberberg, R. Malignant lymphoid tumors in orchidectomized mice receiving hypophyseal and ovarian grafts at various ages. *Proc. Soc. Exper. Biol. & Med.*, 1949, 72, 547-550.
11. Lipschutz, A. Les hormones préhypophysaires dans la tumorigénèse expérimentale. *Ann. d'endocrinol.*, 1952, 13, 9-23.
12. Gorbman, A. Functional and structural changes consequent to high dosages of radioactive iodine. *J. Clin. Endocrinol.*, 1950, 10, 1177-1191.
13. Goldberg, R. C., and Chaikoff, I. L. The cytologic changes that occur in the anterior pituitary glands of rats injected with various doses of  $I^{131}$  and their significance in the estimation of thyroid function. *Endocrinology*, 1950, 46, 91-104.
14. Goldberg, R. C., Chaikoff, I. L., Lindsay, S., and Feller, D. D. Histopathological changes induced in the normal thyroid and other tissues of the rat by internal radiation with various doses of radioactive iodine. *Endocrinology*, 1950, 46, 72-90.
15. Rugh, R. Radioiodine and histopathological effects. *J. Morphol.*, 1951, 89, 457-499.
16. Rugh, R. The mouse thyroid and radioactive iodine ( $I^{131}$ ). *J. Morphol.*, 1951, 89, 323-365.
17. Maloof, F., Dobyns, B. M., and Vickery, A. L. The effects of various doses of radioactive iodine on the function and structure of the thyroid of the rat. *Endocrinology*, 1952, 50, 612-638.
18. Silberberg, R., Silberberg, M., and Dixon, F. J. Obliterating tracheitis, a complication following administration of radioactive iodine. *J. Lab. & Clin. Med.*, 1952, 39, 256-259.
19. Furth, J., Burnett, W. T., Jr., Gadsden, E. L., and Anderson, B. Quantitative assays for TSH in relation to thyrotrophin-secreting pituitary tumors. To be published.
20. Anderson, E., and Bates, R. W. Unpublished data.

21. Bischoff, F., Clarke, G. J., and Epps, C. H. Influence of the thyroid on the resorption of gonadotropic hormones. *Endocrinology*, 1941, 28, 48-52.
22. Furth, J., Gadsden, E. L., and Upton, A. C. Hyperplasia and cystic dilatation of extrahepatic biliary tracts in mice bearing grafted pituitary growths. *Cancer Research*, 1952, 12, 739-743.
23. Gardner, W. U., Allen, E., and Smith, G. M. Hyperplasia and hypertrophy of the mucosa of larger biliary ducts in mice receiving estrogens. *Proc. Soc. Exper. Biol. & Med.*, 1941, 46, 511-513.
24. Cramer, W., and Horning, E. S. Adrenal changes associated with oestrin administration and mammary cancer. *J. Path. & Bact.*, 1937, 44, 633-642.
25. Sobel, H., and Furth, J. Hypervolemia in mice bearing granulosa cell growths; time of onset and some associated physiological and chemical changes. *Endocrinology*, 1948, 42, 436-447.
26. Furth, J. Conditioned and autonomous neoplasms: A review. *Cancer Research*, 1953, 13, 477-492.
27. Morris, H. P., Dalton, A. J., and Green, C. D. Malignant thyroid tumors occurring in the mouse after prolonged hormonal imbalance during the ingestion of thiouracil. *J. Clin. Endocrinol.*, 1951, 11, 1281-1295.
28. Dickie, M. M., and Woolley, G. W. Spontaneous basophilic tumors of the pituitary glands in gonadectomized mice. *Cancer Research*, 1949, 9, 372-384.
29. Dunning, W. F., Curtis, M. R., and Segaloff, A. Strain differences in response to diethylstilbestrol and the induction of mammary gland and bladder cancer in the rat. *Cancer Research*, 1947, 7, 511-521.
30. Furth, J., Gadsden, E. L., and Upton, A. C. ACTH secreting transplantable pituitary tumors. *Proc. Soc. Exper. Biol. & Med.*, 1953, 84, 253-254.
31. Greene, H. S. N. A conception of tumor autonomy based on transplantation studies: a review. *Cancer Research*, 1951, 11, 899-903.
32. Laqueur, G. L. Personal communication.

#### LEGENDS FOR FIGURES

FIG. 1. Characteristic appearance of a mouse with a pituitary tumor induced by  $I^{131}$ .

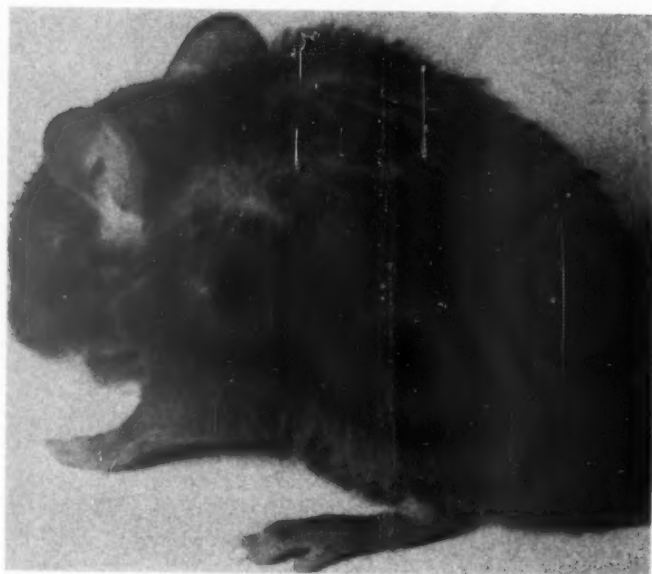
FIG. 2. Two primary pituitary tumors (4077 and 5001) induced by  $I^{131}$  with a normal pituitary body in the center. Mouse 4077 died 12½ months after subcutaneous injection of 50  $\mu$ c. of  $I^{131}$ .







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Figures 3 to 8 illustrate dependent tumor strain 3.

- FIG. 3. The large cells shown were rarely present in first generation grafts. They vanished on subsequent passages and their character is not known.  $\times 450$ .
- FIG. 4. Same tumor as in Figure 3, showing the numerous intranuclear inclusions frequently present in thyrotrophin-secreting dependent tumors.  $\times 470$ .
- FIG. 5. Gland-like structures occasionally seen in primary tumors and first generation grafts.  $\times 470$ .
- FIG. 6. Characteristic appearance of dependent tumor cells. There is uniformity in shape and size of cells and the cytologic features of cancer cells are lacking. Martins and Mallory's trichrome stain.  $\times 450$ .
- FIG. 7. Early growth of a dependent tumor with lack of degenerative changes. Cavernous sinusoids, as in this field, were numerous.  $\times 120$ .
- FIG. 8. Metastasis to regional lymph node.  $\times 470$ .

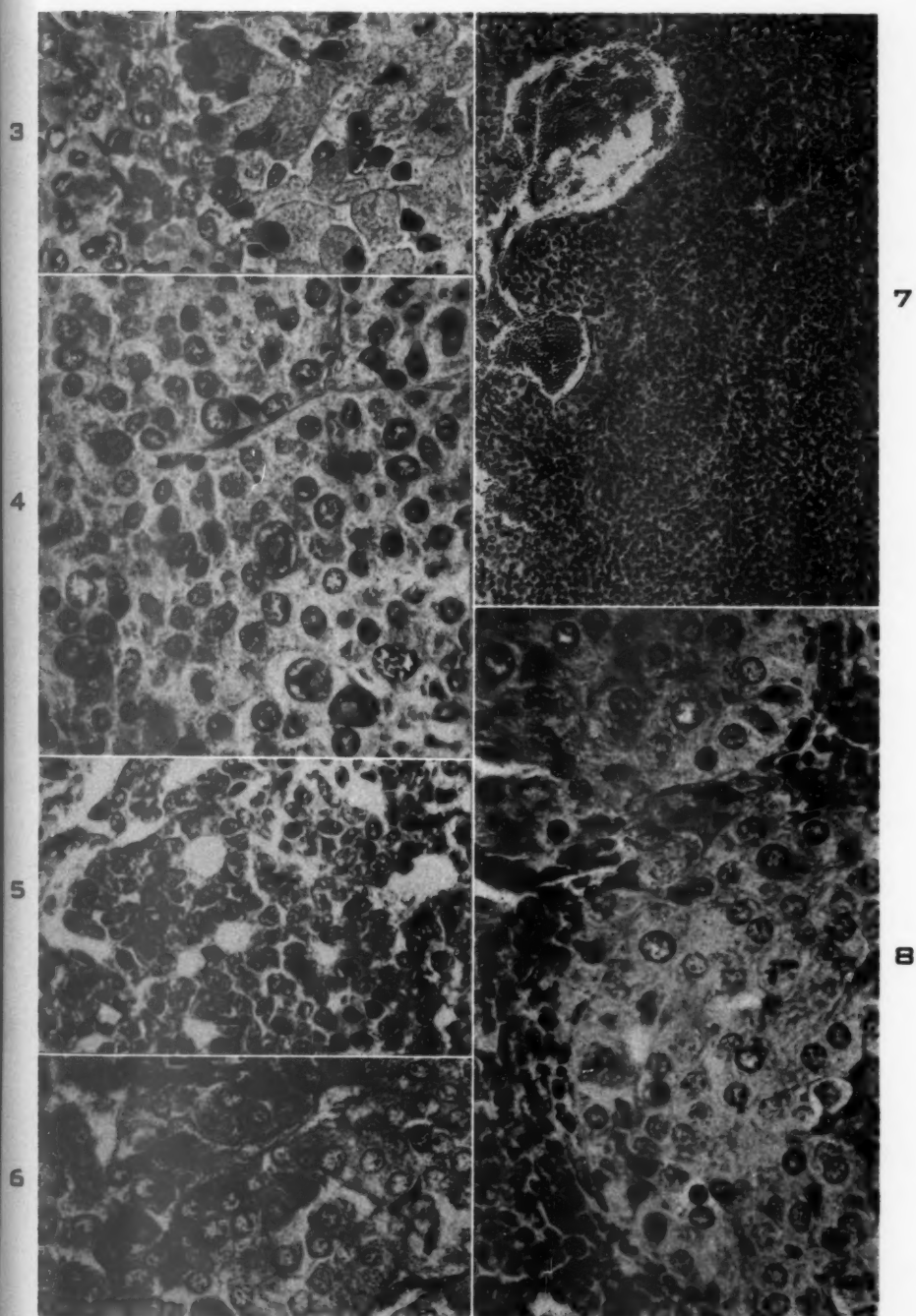


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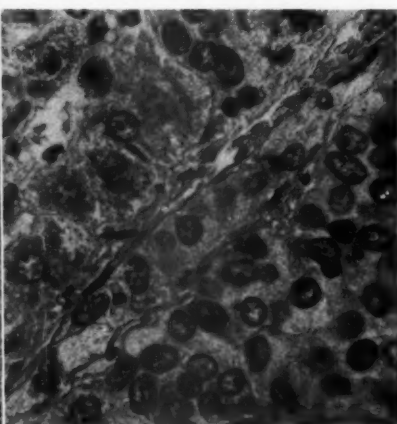
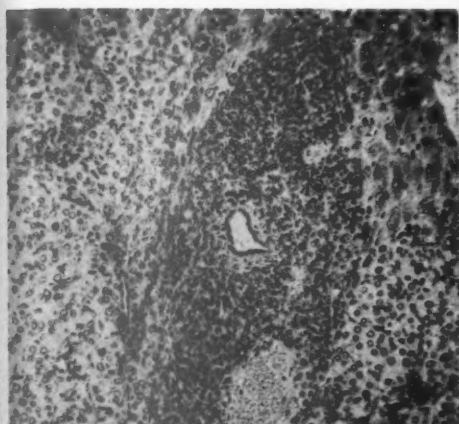


- FIG. 9. Metastasis to liver following intrasplenic graft (strain 3D), with malignant lymphoma.  $\times 120$ .
- FIG. 10. Same as Figure 9 showing, at magnification  $\times 470$ , lymphomatous infiltration about a bile duct.
- FIG. 11. Same as Figure 9 showing, at magnification  $\times 470$ , liver cells and tumor cells.
- FIG. 12. Malignant lymphoma and dependent pituitary tumor cells following subcutaneous graft (strain 124D).  $\times 120$ .
- FIG. 13. Autonomous pituitary tumor graft (strain 3A) in the right thigh. Extensive metastases to lymph nodes. Atrophy of uterine horn.
- FIG. 14. Tumor 3A graft. Extensive metastases to retroperitoneal lymph nodes. Atrophy of seminal vesicles.
- FIG. 15. Tumor 3A graft. Extensive metastases in retroperitoneal lymph nodes and ovaries.

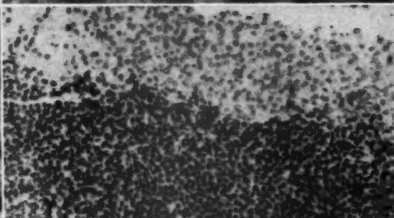
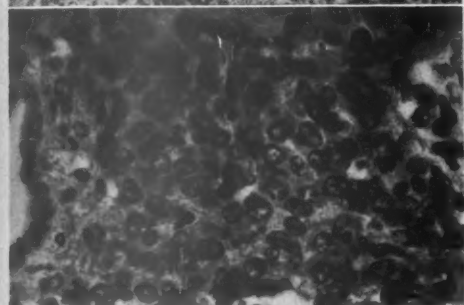




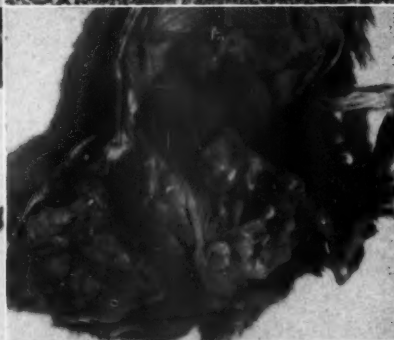




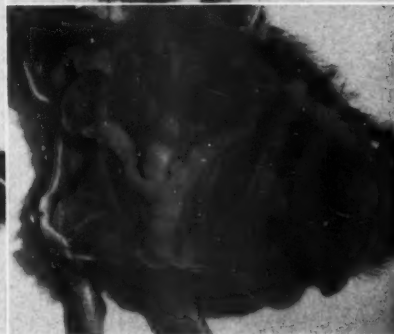
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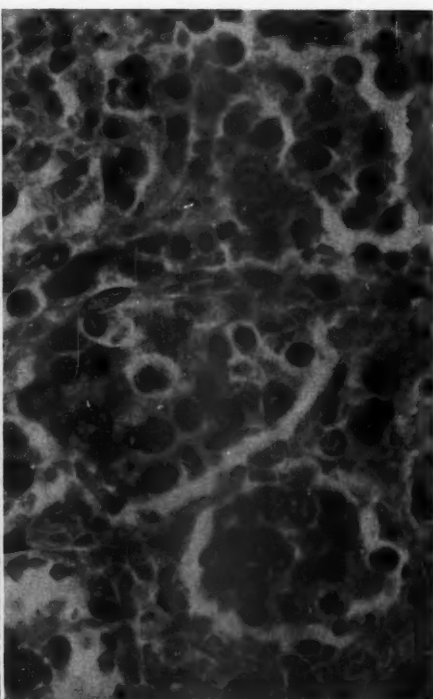
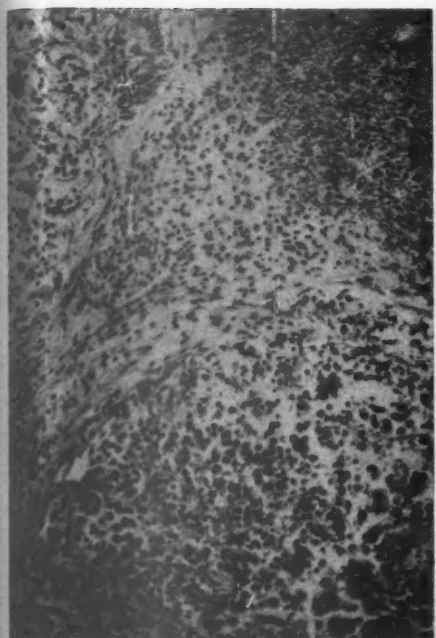
Figures 16 to 19 illustrate the autonomous tumor strain 3A.

FIG. 16. Extensive hemorrhage, moderate fibrosis, and necrosis.  $\times 120$ .

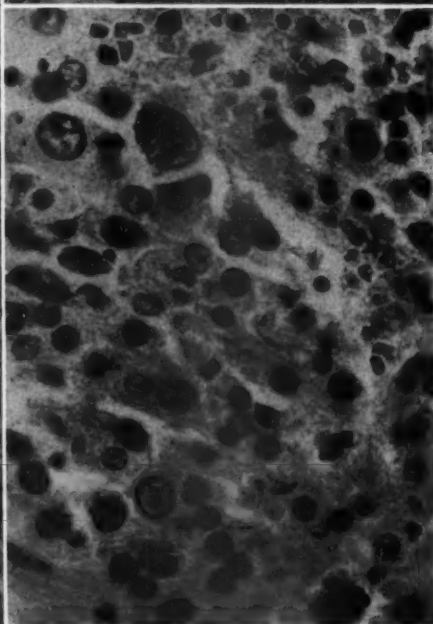
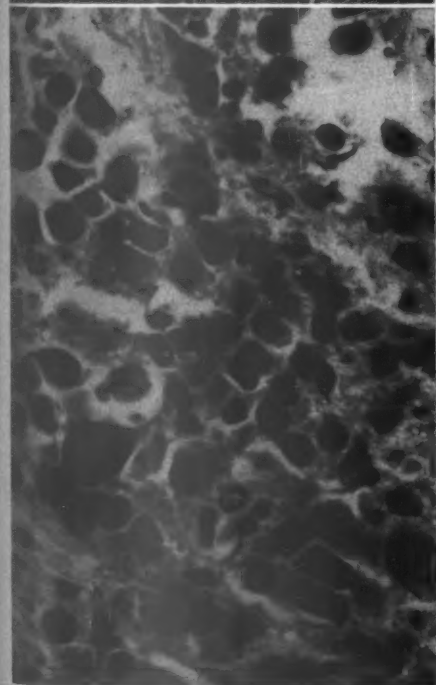
FIGS. 17 to 19. Several fields of the autonomous tumor shown in Figure 16, at magnification  $\times 470$ , exhibiting features of a malignant growth.







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FIGS. 20 and 21. Normal thyroid gland.  $\times 33$  and 250.

FIGS. 22 and 23. Near-complete radiothyroidectomy. These atypical thyroid cells are apparently incapable of regeneration. This mouse died 223 days after administration of  $I^{131}$ .  $\times 33$  and 470.

FIGS. 24 and 25. Diffuse stimulation of the thyroid gland by late autonomous pituitary tumor. The animal died 7 months following graft of a tumor 3D.  $\times 33$  and 470.

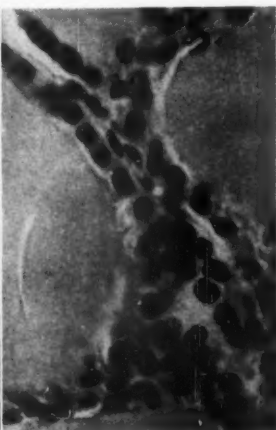
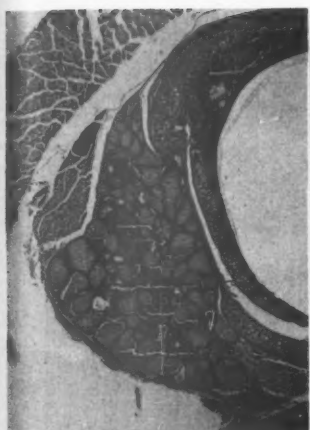
FIG. 26. A thyroid adenoma following near-complete destruction of this organ with 100  $\mu$ c. of  $I^{131}$ ; this animal had a pituitary tumor of about 4 by 6 mm., 14 months following administration of  $I^{131}$ .  $\times 120$ .



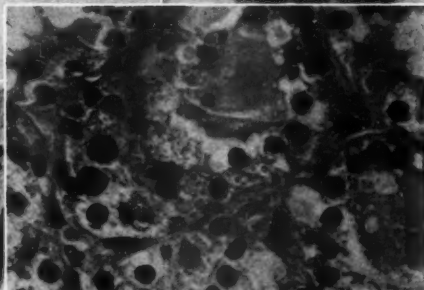
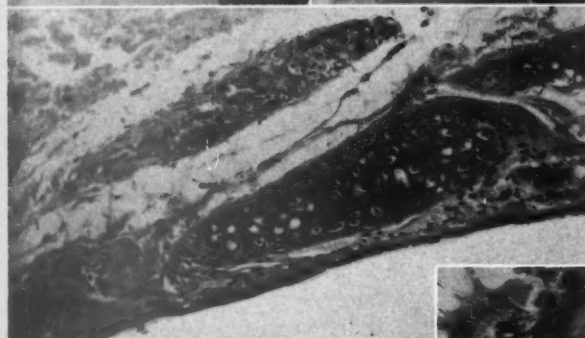




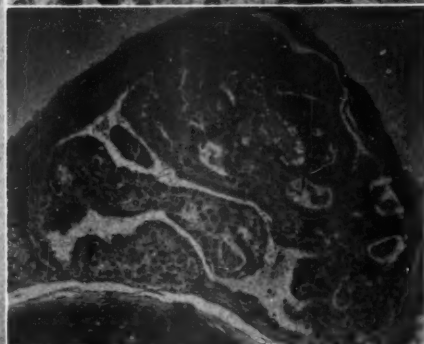
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FIG. 27. Cross section of normal trachea.  $\times 120$ .

FIG. 28. Incidental changes 367 days after radiothyroidectomy. Arterial stenosis; fibrosis at site of the thyroid gland. Calcification of cartilage. Fibrosis of submucosa with absence of mucous glands.  $\times 120$ .

FIG. 29. Stenosing tracheitis 300 days after radiothyroidectomy.  $\times 33$ .

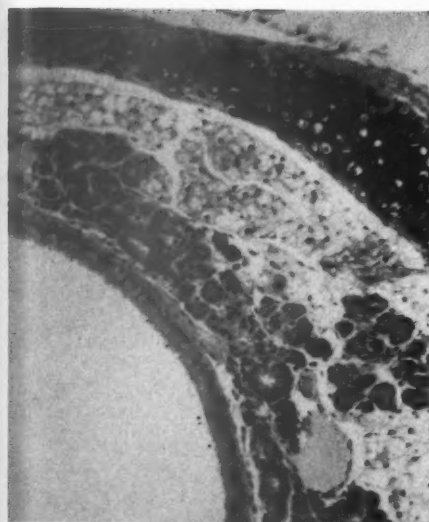
FIGS. 30 and 31. Partial destruction of the parathyroid gland following radiothyroidectomy. Stenosing arteritis; adjacent area of fibrosis marks the site of the thyroid gland.  $\times 120$ .

FIGS. 32 and 33. Arterial changes 8 days following administration of  $270 \mu\text{c}$ . of  $\text{I}^{131}$ .  $\times 120$ .

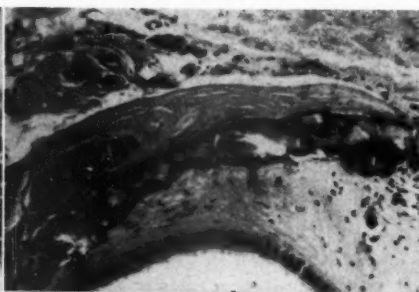
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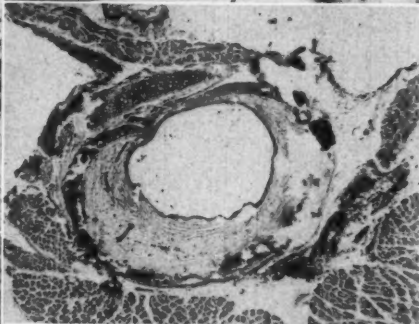
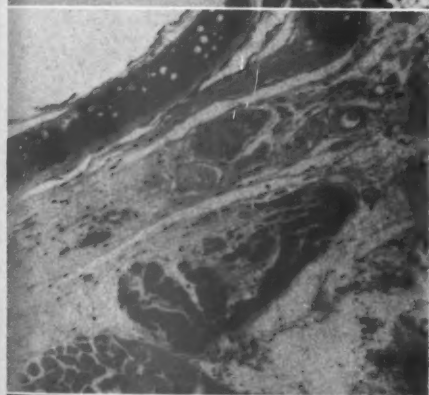




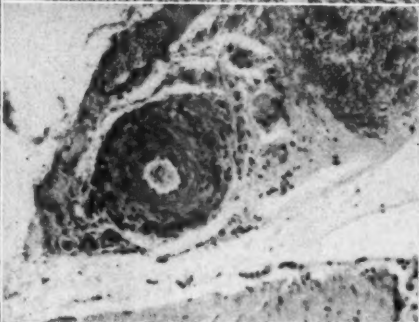
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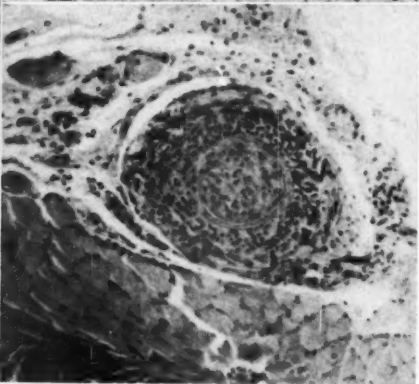
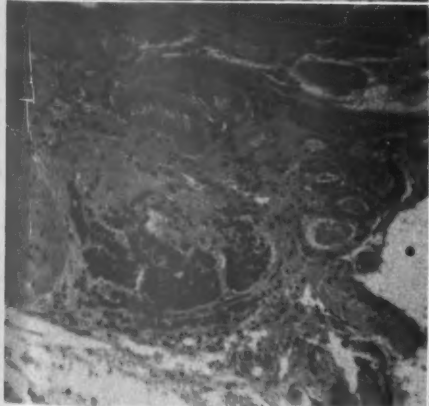




FIG. 34. Enormous enlargement of the thyroid gland with adenomas caused by grafted late autonomous pituitary tumor (strain 3).

FIG. 35. Normal thyroid gland shown for comparison with Figure 34.

FIGS. 36 and 37. Hyperplasia of the thyroid gland with adenomas, in a mouse bearing a late autonomous tumor (strain 3).  $\times 120$  and  $240$ .

FIG. 38. Enormous enlargement of the thyroid gland with numerous adenomas, in a mouse bearing a late autonomous pituitary tumor (strain 3). This animal died 7 months after the graft.  $\times 33$ .

FIG. 39. Invasion of a blood vessel by thyroid tissue in a mouse with greatly enlarged adenomatous thyroid gland caused by a late autonomous pituitary graft (strain 3). Necropsy 15 months after the tumor graft.  $\times 120$ .

FIGS. 40 and 41. Intramuscular thyroid grafts showing evidence of stimulation, in mice bearing grafted autonomous pituitary tumors (3A).  $\times 120$ .

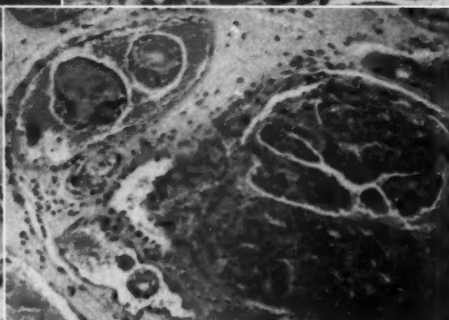
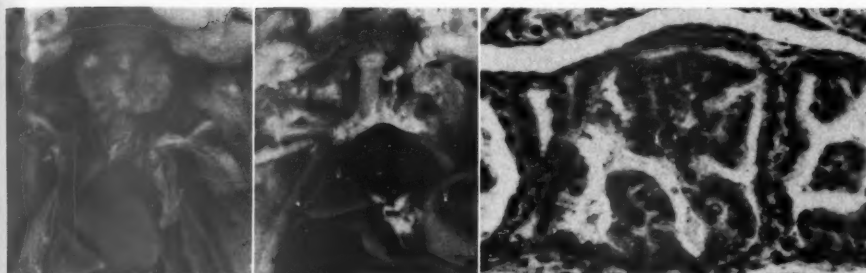




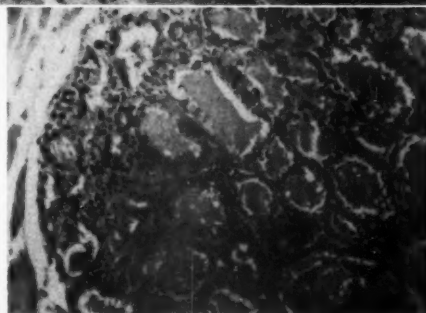
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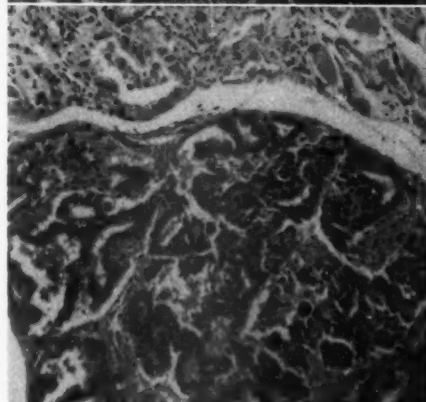
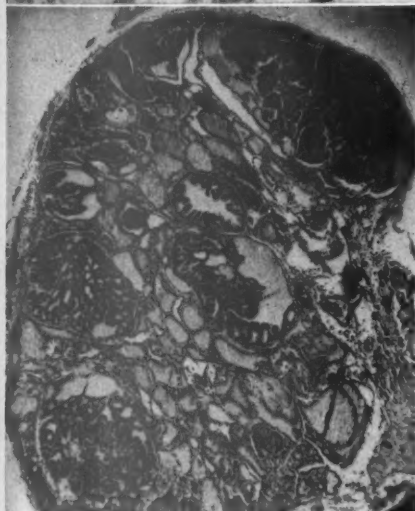
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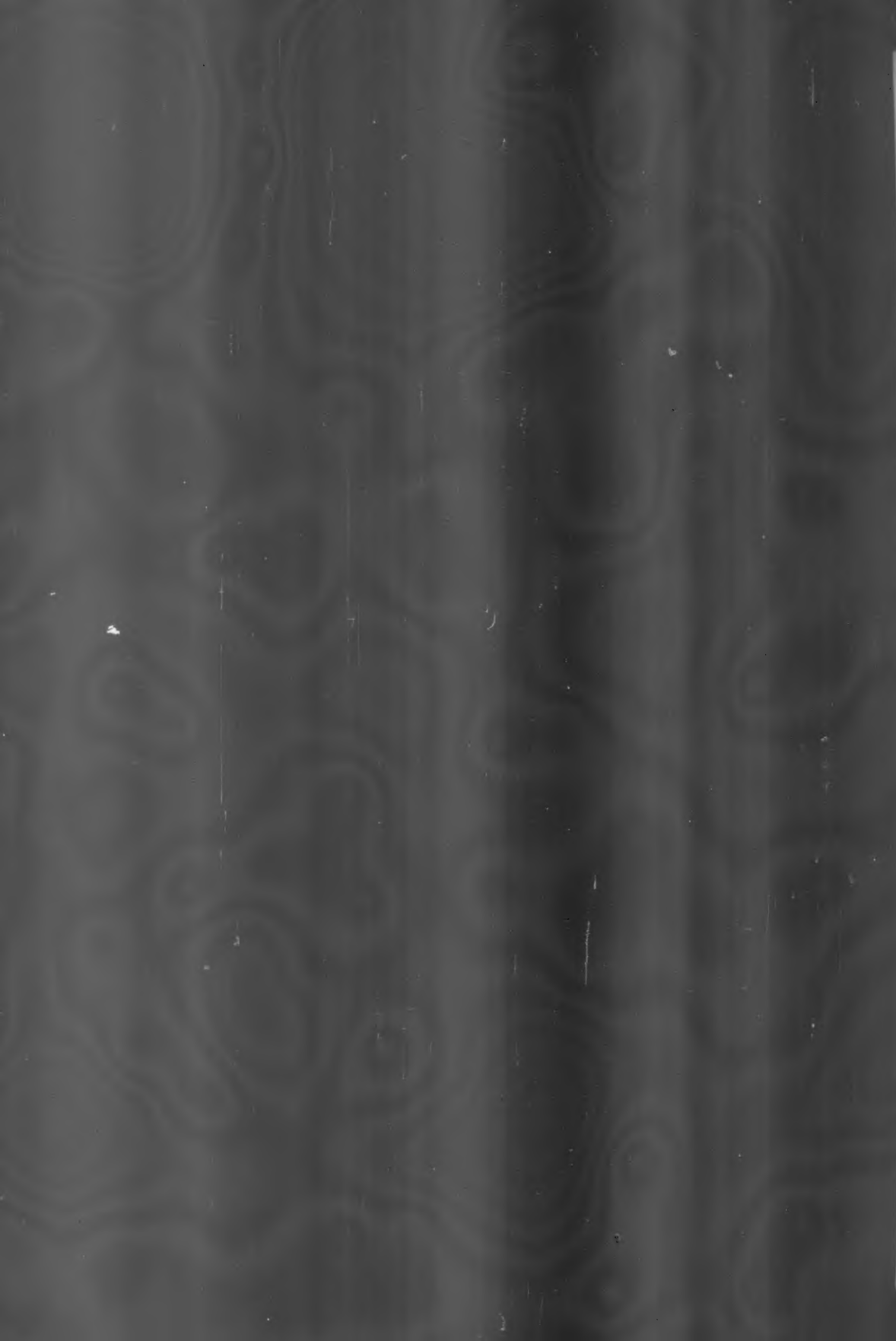
FIGS. 42 and 43. Intrapulmonary thyroid tissue following intravenous injection of thyroid cells in a mouse bearing an autonomous pituitary tumor (strain 3A).  $\times 120$  and 450.

FIGS. 44 and 45. Intrapulmonary thyroid tissue following intravenous injection of thyroid cells in a mouse bearing an autonomous pituitary tumor (strain 19A).  $\times 450$ .

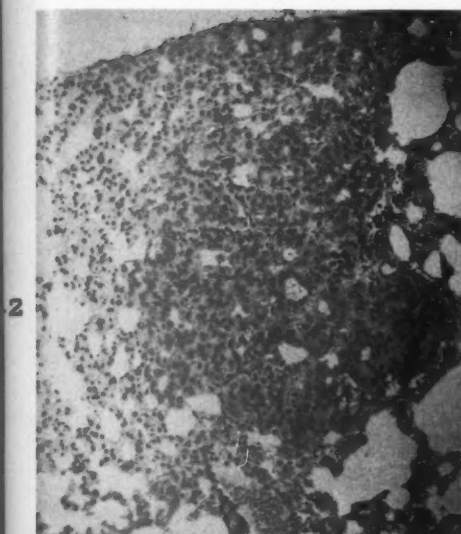
FIG. 46. Massive hepatic necrosis in a mouse having thyroid tissue grafted in the lung and an autonomous pituitary tumor in the thigh. (Most mice of this group died suddenly, probably of thyrotoxicosis).  $\times 120$ .

FIG. 47. Cleft-like spaces in the acinar cells of the pancreas of a mouse bearing an autonomous pituitary tumor (3A).  $\times 450$ .

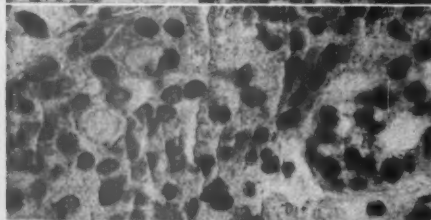




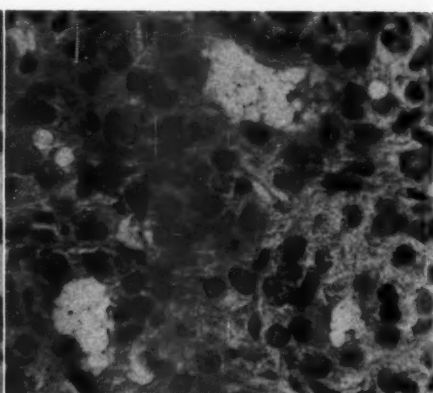
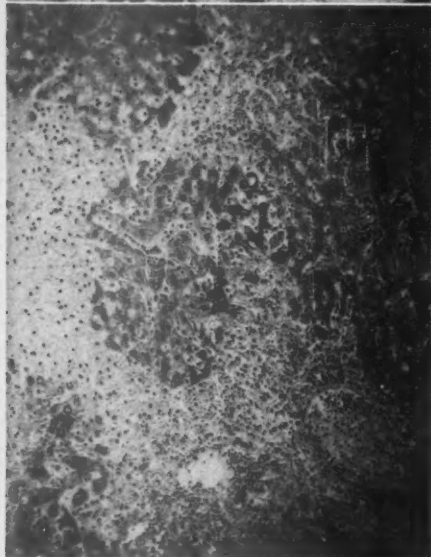




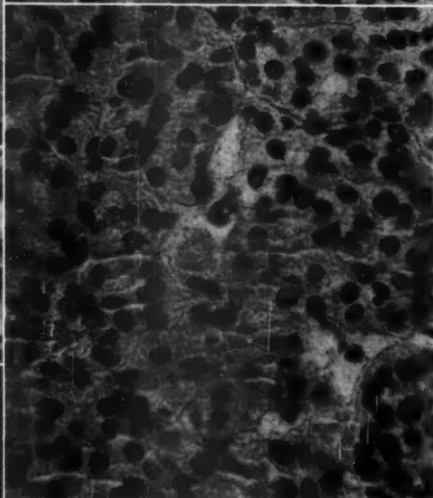
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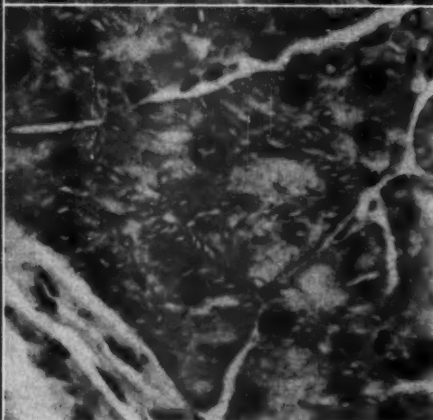
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47

FIG. 48. Two mice bearing grafted dependent tumors (not seen in the picture) with cystic dilatation of the extrahepatic biliary tracts.

FIGS. 49 and 50. Hyperplasia and dilatation of the hepatic duct in a mouse bearing a grafted dependent tumor.  $\times 33$ .

FIG. 51. Hyperplasia and dilatation at ampulla of Vater. Arrow points to the patent lumen near the orifice.  $\times 33$ .

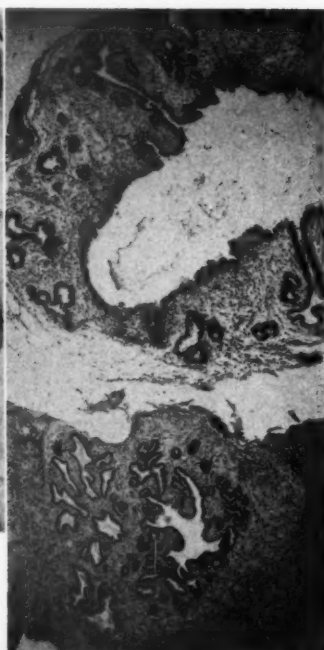
FIG. 52. Atrophic ovary with lutein cells in a mouse with a primary pituitary tumor induced by radiothyroidectomy.  $\times 120$ .







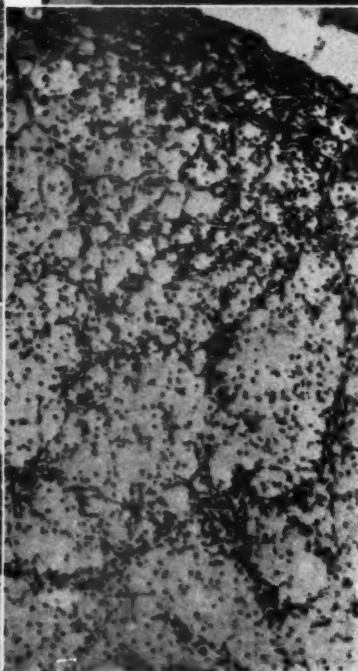
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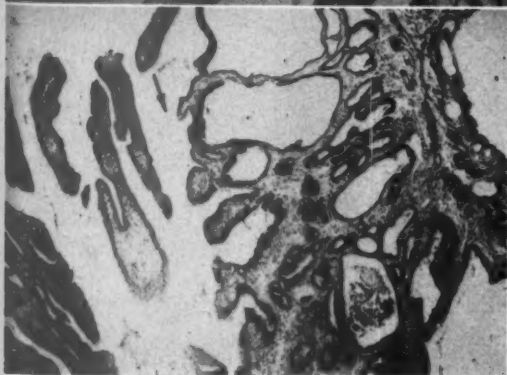
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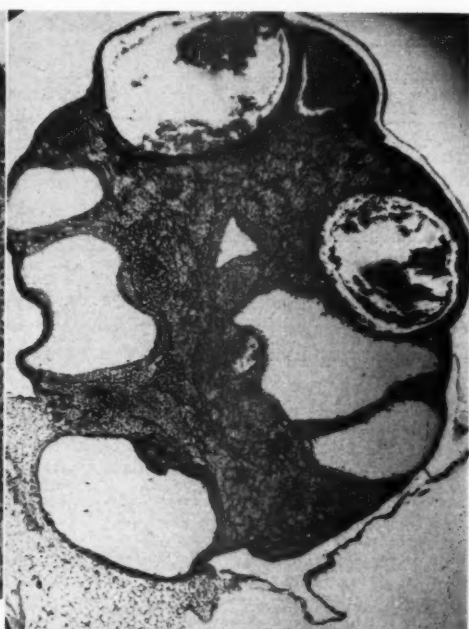
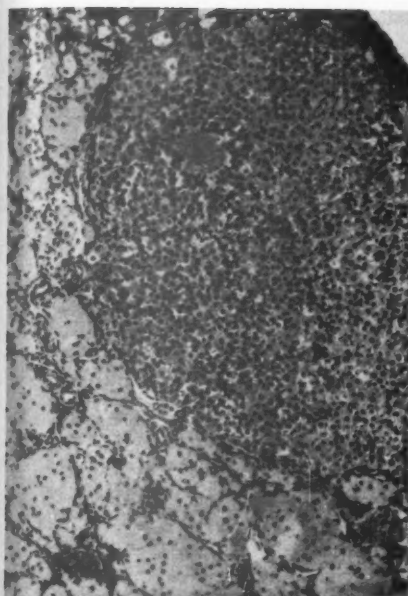


- FIG. 53. Atrophic ovary with a lutein body (microluteoma?) in a mouse with pituitary tumor induced by radiothyroidectomy.  $\times 120$ .
- FIG. 54. Extensive gonadal stimulation ("A-Z reaction") in a radiothyroidectomized mouse bearing a grafted dependent tumor. Estrogenic type of uterine stimulation (strain 3D).
- FIG. 55. Gonadal stimulation ("A-Z reaction") in a radiothyroidectomized mouse bearing a grafted dependent tumor. Cystic dilatation of the uterine horn (strain 3D).
- FIGS. 56 and 57. Greatly stimulated ovaries ("A-Z reaction") in mice bearing grafted dependent tumors; phase of follicular ripening with hemorrhage and luteinization of stroma cells.  $\times 33$  and  $120$ .

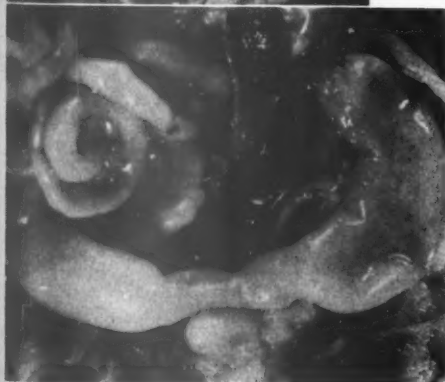








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FIG. 58. Normal adrenal gland of an adult mouse.  $\times 120$ .

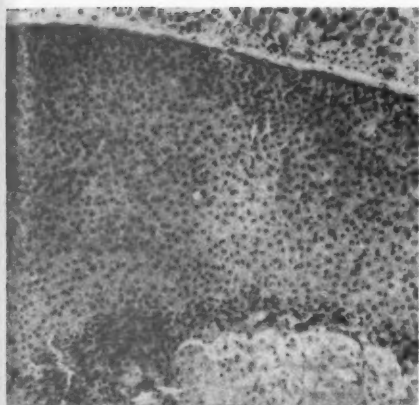
FIG. 59. Atrophic adrenal cortex of a mouse with primary pituitary tumor induced by radiothyroidectomy.  $\times 120$ .

FIGS. 60 and 61. Replacement of the reticular zone of the adrenal cortex with "foam" cells; a characteristic change secondary to dependent tumors.  $\times 120$  and  $470$ .

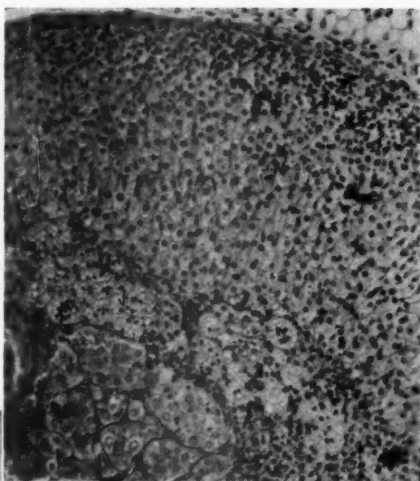
FIGS. 62 and 63. Characteristic lipid degeneration in the region of the reticular zone of the adrenal gland in a normal mouse bearing a late autonomous tumor.  $\times 120$  and  $470$ .



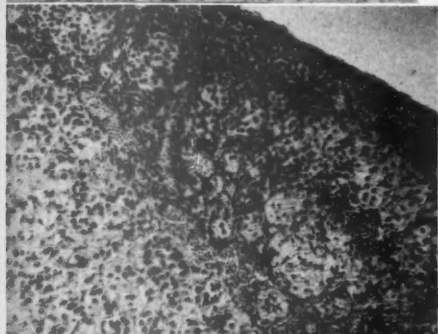




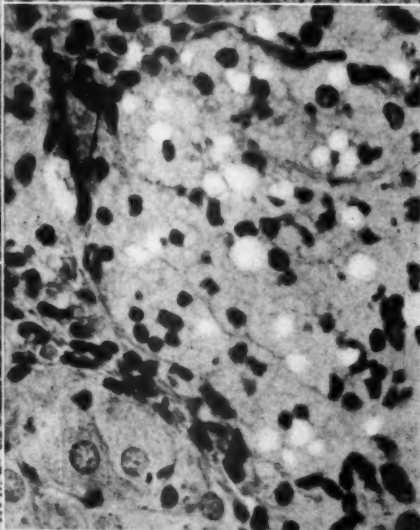
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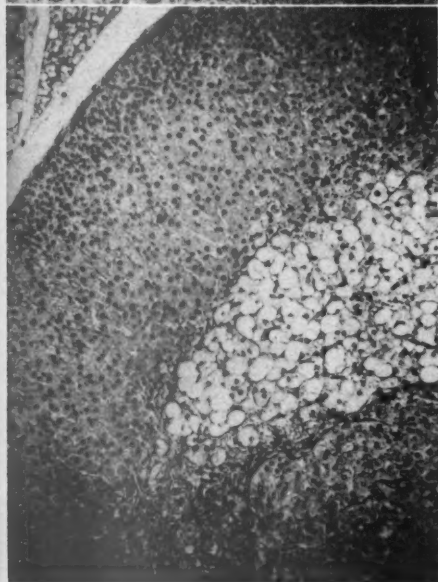
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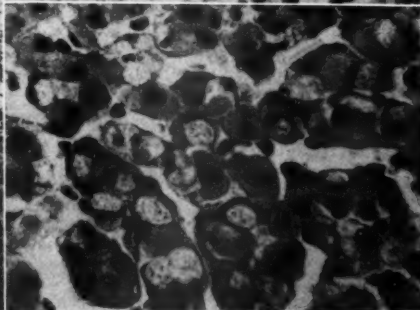
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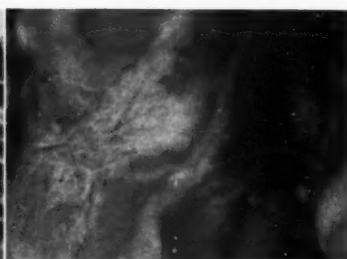
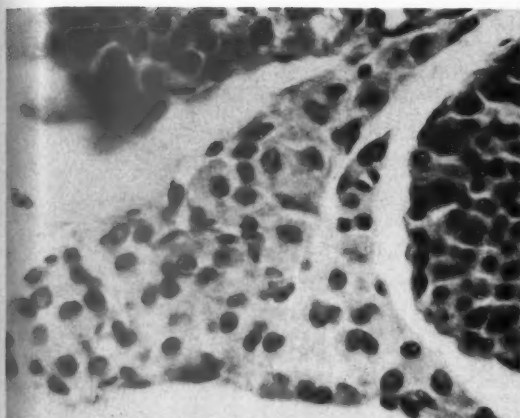
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- FIG. 64. Hyperplasia of Leydig cells in a radiothyroidectomized mouse bearing a dependent tumor.  $\times 470$ .
- FIG. 65. Hyperplasia of the mammary gland of a radiothyroidectomized mouse bearing a grafted dependent tumor (strain 19D).
- FIG. 66. Hyperplasia of the mammary gland of a radiothyroidectomized mouse bearing a primary pituitary tumor.  $\times 120$ .
- FIG. 67. Grafted dependent pituitary tumor with hyperplasia of the adjacent mammary gland.  $\times 120$ .
- FIGS. 68 and 69. Tumor of the submaxillary gland with osteogenesis in a mouse 345 days following radiothyroidectomy.  $\times 120$  and  $470$ .

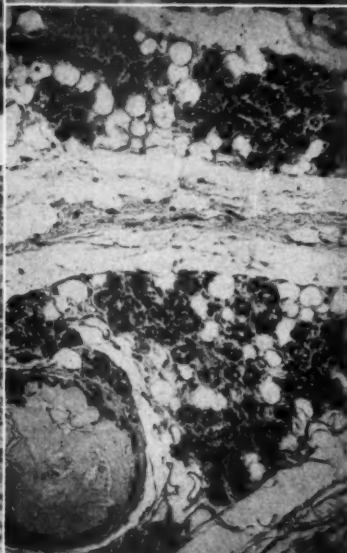
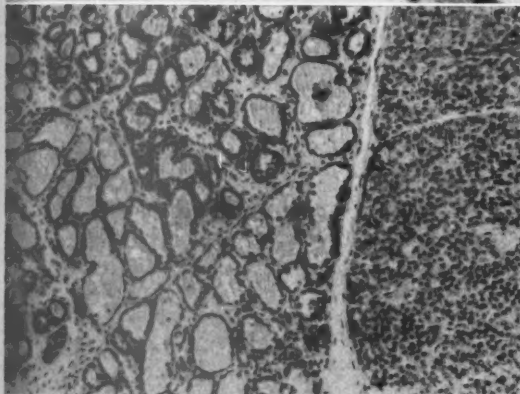




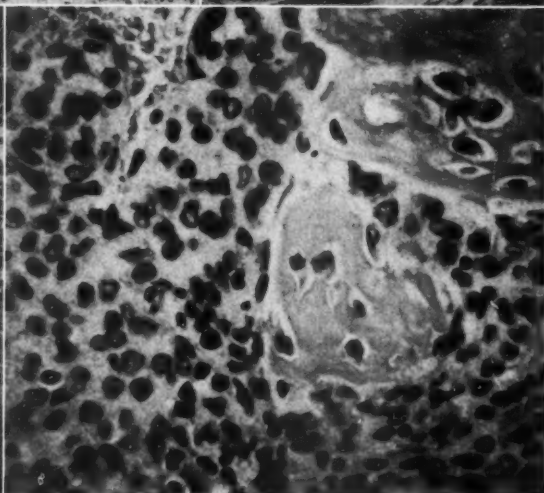
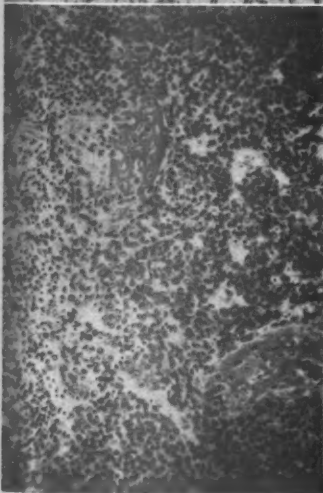




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## ACINIC CELL ADENOCARCINOMA OF THE PAROTID GLAND

### REPORT OF TWENTY-SEVEN CASES \*

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The purpose of this paper is to record the clinicopathologic features of 27 examples of heretofore rarely encountered or described acinic cell adenocarcinomas of the parotid gland. Twenty-two of these were found among about 900 major salivary gland tumors treated at Memorial Hospital since 1930. Five additional cases submitted from other institutions because of their unusual nature are included.

### REVIEW OF LITERATURE

The term adenoma has been employed to designate many tumors of salivary glands. At present we employ it only for the oxyphilic granular cell tumor and papillary cystadenoma lymphomatosum. A brief review of the literature on adenomas will follow; little mention will be made of the oxyphilic granular cell adenoma which has been amply reviewed by Stump,<sup>1</sup> Meza-Chávez,<sup>2</sup> and others, or of the papillary cystadenoma lymphomatosum recently reviewed by Thompson and Bryant.<sup>3</sup>

In 1892, Nasse<sup>4</sup> described four parotid adenomas composed of cells which resembled the normal acinic cells. Since that time several additional reports of salivary gland adenomas have appeared. However, it is nearly impossible to be certain of the exact nature of many of these tumors; the descriptions or illustrations are difficult to interpret, and authors have disagreed as to the correct designation of several of these lesions. Lecène<sup>5</sup> described a cystic adenoma and cited the writings of Küttner,<sup>6</sup> Ribbert,<sup>7</sup> and Lexer.<sup>8</sup> Lambret and Pélissier<sup>9</sup> described an adenoma which they believed to be of canalicular origin and stated that they thought the cases of Lexer, Ribbert, and Lecène were of similar origin. They stated that Nasse had been the first to report a case of adenoma of the parotid gland and they believed earlier reports of adenomas were actually descriptions of mixed tumors. Lambret and Pélissier stated that adenomas may arise either in the gland (intra-

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glandular type) or outside the gland proper (extraglandular type) and they believed their case to be extraglandular. From the point of view of origin they stated that adenomas may arise either from the acini (the acinic form), such as the fourth case of Nasse, or from the system of excretory tubes (canalicular form), such as their case and the cases of Lexer, Ribbert, and Lecène.

In 1924 Masson<sup>10</sup> described an adenoma that he stated to be of acinic cell origin which clearly resembles some of the cases herein reported. Schutz,<sup>11</sup> in 1926, also reported a case which he stated appeared to be of acinic origin. Hückel<sup>12,13</sup> recorded three adenomas which he believed arose from the glandular epithelium of the parotid gland. Stump<sup>1</sup> has included one of these cases as an onkocytic adenoma in his listing of cases of this type of tumor, but Hückel believed his three tumors to be similar.

Lang<sup>14</sup> gave a short account of salivary gland adenomas and stated that they may be of ductal or glandular epithelial origin. He cited Lambret and Péliissier<sup>9</sup> as having divided adenomas into acinic and canalicular types. Franssen<sup>15</sup> reported a case of a parathyroid-like tumor in the parotid gland which he believed was derived from parotid epithelial cells. Alhbom<sup>16</sup> recorded two adenomas, one of which (case 91) has been accepted as an onkocytic adenoma by Stump.<sup>1</sup> The second (case 59) may represent an acinic cell tumor. Lkyd<sup>17</sup> reported a case of adenoma (case 9), the photomicrograph of which suggests acinic origin. In 1948 Godwin and Colvin<sup>18</sup> reported an adenoma which they believed of acinic origin; this tumor has not recurred after 5 years. Recently Bauer and Bauer<sup>19</sup> reported three cases of parotid adenoma, two of acinic type and the third composed of ducts and acini. Buxton, Maxwell, and French<sup>20</sup> reported several parotid tumors designated as serous cell adenoma and adenocarcinoma which are similar to the cases herein recorded.

#### PATHOLOGIC FINDINGS

Grossly, the acinic tumor may resemble superficially a mixed tumor, but close inspection will reveal a difference. The acinic tumor does not present the moist myxomatous appearance of the mixed tumor. It is encapsulated, round or oval, and on cut surface is lobulated, friable, and generally homogeneously glistening grayish white. Occasionally there may be necrotic foci and cysts. The recurrent lesions may show multiple foci which are similar to those of mixed tumors (Figs. 1 and 2).

Microscopically, the capsule is usually thin and, where contiguous parotid gland is present, usually shows some loss of acini with mild

chronic inflammation. The tumors, though more often solid, may be cystic (Fig. 3), and the cells may have a glandular (Fig. 4) or formless arrangement. The stroma and blood vessels are sparse. Numerous intercellular vacuoles are frequent and suggest secretory material (Fig. 5). The individual granulated cell composing the tumor may be the size of, or much larger than, the normal acinic cell. It contains granules that are histologically and histochemically similar to the granules of normal acinic cells (Figs. 6 and 7); the cytoplasmic granules are basophilic. The cell membranes are distinct and the nuclei uniformly small and dark (Fig. 8). The cells are occasionally clear. In some tumors there are cells which are not granulated and do not resemble acinic cells but appear more closely akin to the cells of the intercalated duct or those at the junction of the acinus and intercalated duct.

No mixed tumor components or areas suggestive of other types of commonly recognized salivary gland tumors have been found, even on serial sectioning of portions of several of these tumors. Mucicarmine stains of several lesions have been negative, which effectively removes them from the muco-epidermoid group of tumors. An occasional tumor contains a prominent lymphoid stroma (Fig. 9) or foci of calcification. Fat stains have revealed only fine droplets which are occasionally intracellular but generally intercellular. Glycogen staining was positive in a few areas in two cases. Periodic acid-Schiff's staining is positive in normal parotid acinic cells and many of the granulated cells of the tumors gave positive results by this method; a histochemical similarity is demonstrated thereby (Fig. 10). Where material is present in the intercellular spaces it stains positively with the periodic acid-Schiff procedure.

Metastasis to lymph nodes was found in one (case 3) of several cases in which specimens from neck dissection were studied; the deposits were found in two contiguous lymph nodes. Metastasis to distant organs has occurred in several cases, although, based on histologic appearance alone, this trait would not have been expected. An example of the rather innocuous appearance of a pulmonary metastasis is shown in Figure 11.

It is our belief, based on histologic resemblance, absence of other recognizable tumor types in multiple sections, foci of apparent serous secretions, and the similarity of the results of periodic acid-Schiff's staining in normal and in cancerous cells, that these tumors arise from the acinic cells.

#### CLINICAL OBSERVATIONS

Clinical observations upon the 27 cases are summarized in Table I. Clinically, the lesion closely simulates a mixed tumor. All of these



TABLE I  
Clinical Data for Twenty-seven Cases of Acinic Cell Adenocarcinoma of the Parotid Gland

Case*	Age at onset	Sex	Parotid gland	Interval before treatment	Pre-viously treated	Treatment	Tumor size in cm.	Recurrence	Follow-up	Metastasis
1. A.K.	17	M	Left	3 yrs.	Yes	Excision, radon	2 x 1.2 x 1	No	NEN† after 9 yrs.	No
2. M.S.	45	F	Right	1 yr.	Yes	Excision, radiation		Several	Died after 24 yrs. NEN?	No
3. O.W.	28	F	Right	16 yrs.	Yes	Radiation, excision, neck dissection†		Several	DON‡ after 8 yrs.	Lung, subcutaneous and regional nodes
4. E.H.	54	F	Right	3 yrs.	No	Excision, radiation	4 x 2.5 x 3	No	NEN after 13 yrs.	No
5. G.E.	68	F	Right	6 mos.	Yes	Excision	2.2	No	NEN after 7 yrs.	No
6. B.M.	56	M	Right	4 mos.	No	Excision	3.5 x 2 x 0.7	No	No	No
7. A.L.	53	F	Left	5 mos.	No	Excision	2 x 1.5 x 2	No	NEN after 1 yr.	No
8. H.B.	53	F	Right	6 mos.	Yes	Excision, neck dissection	3 x 3 x 1.5	Several	LWN   after 3 yrs.	Lung
9. P.H.	51	M	Left	Few weeks	Yes	Radical parotidectomy	6.5 x 4.5 x 4	Several	NEN after 3 yrs.	No
10. G.S.	30	F	Right		Yes	Radical excision, neck dissection	3.5 x 2	Several	Local recurrence	No
11. V.H.	23	F	Left		Yes	Excision, neck dissection		Several	NEN after 30 yrs.	No
12. F.G.	25	F	Right	2-3 yrs.	No	Excision	2.5 x 2 x 1.5	No	NEN after 2 yrs.	No
13. C.B.	62	M	Left	4 yrs.	No	Excision, neck dissection	2.5	Yes	DON after 1.5 yrs.	Lung and bone
14. A.D.	55	F	Left		Yes	Excision	2.0	Several	Recurrence	No
15. J.T.	55	M	Left	6 yrs.	Yes	Excision	2.5 x 2 x 2	One	Too recent	No
16. F.G.	43	F	Right	4 yrs.	Yes	Excision, neck dissection	2.2	Yes	NEN after 8 yrs.	No
17. E.L.	31	F	Left	15 yrs.	Yes	Excision, neck dissection		No	NEN after 2 yrs.	No
18. R.R.	20	M	Left	8 yrs.	No	Excision	2.5	No	Too recent	No
19. E.G.	60	F	Right	4 yrs.	Yes	Excision, radon		One	NEN after 12 yrs.	No
20. H.S.	33	M	Left	6 yrs.	Yes	Excision		Several	NEN after 20 yrs.	No
21. A.S.	18	F	Right	4 yrs.	No	Excision, radon	2.0	Yes	NEN after 15 yrs.	No
22. G.A.	46	F	Right	8 mos.	No	Excision	1.0	No	Too recent	No
23. F.G.	42	F	Left				1.5	Yes	DON	No
24. F.H.	40	M	Left	2 yrs.		Excision		No	Died of pulmonary embolism after 2 mos.	No
25. N.N.	64	F		2 mos.				Yes		
26. A.S.	26	M						Yes	Pulmonary metastasis excised	Lung
27. P.P.	43	M	Left					Yes		

\* Cases 1 to 22 are from the files of Memorial Hospital, New York City; cases 23 to 27 were treated elsewhere and slides submitted to Memorial Hospital.

† Removal of the sternomastoid and omohyoid muscles and submaxillary gland, internal jugular vein, and associated lymph nodes.

‡ NEN = No evidence of neoplasm. § DON = Died of neoplasm. || LWN = Living with neoplasm.

tumors thus far recognized have arisen in the parotid glands; however, occurrence in other salivary glands is to be expected.

The time elapsing between discovery of the tumor by the patient and definitive medical care varied from a few weeks to 16 years. Seventeen of the 27 patients were women. The age range at the date of onset of symptoms was 17 to 68 years. Recurrence was encountered in about 50 per cent of the patients. Many of these patients had had several recurrent lesions at various intervals over long periods.

Five of the patients are dead. One died without apparent residual neoplasm, three died of the neoplasm, and one died of pulmonary embolism 2 months following operation. Three patients are living with the neoplasm and for three the status is indeterminate. Metastasis occurred in two of those dying of the disease. Pulmonary metastases have been found in two of the three patients living with the neoplasm. The facial nerve was not impaired prior to initial treatment but has been impaired in patients suffering recurrence.

#### TREATMENT

Treatment has been largely surgical. It appears that an excision of the tumor with a margin of parotid gland (subtotal parotidectomy), performed with care not to rupture the capsule, constitutes the best method of treatment. Because of the infrequency of lymph node involvement in the material studied thus far, neck dissection does not appear to be indicated.

#### SUMMARY

The clinicopathologic features of 27 cases of acinic cell adenocarcinoma of the parotid gland have been recorded.

These tumors occurred slightly more commonly in females, and most frequently in the fourth and sixth decades. Clinically, they simulated mixed tumors and generally had a long course with frequent recurrences. Metastases to lymph nodes were encountered in one instance and to other organs in four cases. These occurred in instances in which the histologic pattern of the primary lesion would not lead one to expect such a course. Treatment of the primary tumor appears to be excision with a good margin and an intact capsule.

We are indebted to Dr. A. W. Wright, Albany, N.Y., for cases 23 and 26; to Dr. T. O. Winship, Washington, D.C., for case 25; to Dr. A. C. Williams, Jacksonville, Fla., for case 27; to Dr. T. E. McQuade, Coxsackie, N.Y., for case 24; and to the Surgical Staff of Memorial Hospital for the remainder. Leon Z. Saunders, D.V.M., kindly translated the German and French references. The photographs were prepared by Mr. Robert F. Smith, Brookhaven National Laboratory.

## REFERENCES

1. Stump, D. J. Onkocytic adenoma of the salivary glands. *Arch. Path.*, 1949, **48**, 287-296.
2. Meza-Chávez, L. Oxyphilic granular cell adenoma of the parotid gland (oncocytoma). Report of five cases and study of oxyphilic granular cells (oncocytes) in normal parotid glands. *Am. J. Path.*, 1949, **25**, 523-547.
3. Thompson, A. S., and Bryant, H. C., Jr. Histogenesis of papillary cystadenoma lymphomatosum (Warthin's tumor) of the parotid salivary gland. *Am. J. Path.*, 1950, **26**, 807-849.
4. Nasse, D. Die Geschwülste der Speicheldrüsen und verwandte Tumoren des Kopfes. *Arch. f. klin. Chir.*, 1892, **44**, 233-302.
5. Lecène, P. Adénomes et kystes de la parotide. *Rev. de chir., Paris*, 1908, **37**, 1-17.
6. Küttner, H. In: Bergmann, E. v., Bruns, P. v., and Mikulicz, J. Handbuch der praktischen Chirurgie. F. Enke, Stuttgart, 1903, ed. 2, 1, 643. (Cited by Lecène.<sup>5</sup>)
7. Ribbert, H. Geschwulstlehre für Ärzte und Studierende. F. Cohen, Bonn, 1904, p. 394. (Cited by Lecène.<sup>5</sup>)
8. Lexer. Cited by Lecène.<sup>5</sup>
9. Lambret, O., and Pélissier, M. Adénomes de la parotide. *Écho méd. du Nord*, 1911, **15**, 317-324.
10. Masson, P. Atlas du Cancer. Assoc. Franç. p. l'Étude du Cancer, 1922-1931, Troisième fascicule et quatrième fascicule, Sept., 1924. Tumeurs des glandes annexes des muqueuses de la face et du cou. VII Serie A, Plate I, Figs. A, B, C, D.
11. Schutz, C. B. Adenoma of the salivary gland. *Am. J. Path.*, 1926, **2**, 153-157.
12. Hüchel, R. Eine ungewöhnliche Adenomform der Parotis. *Verhandl. d. deutsch. path. Gesellsch.*, 1930, **25**, 342-347.
13. Hüchel, R. Eine parathyreoideaähnliche Geschwulst der Ohrspeicheldrüse. *Zentralbl. f. allg. Path. u. path. Anat.*, 1933, **57**, 57-58.
14. Lang, F. J. Epitheliale Geschwülste der Speicheldrüsen. I. Adenom und Zystadenom. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. Julius Springer, Berlin, 1929, 5, Pt. 2, 127-128.
15. Franssen, R. Eine Parathyreoidea-ähnliche Geschwulst der Ohrspeicheldrüsen. *Zentralbl. f. allg. Path. u. path. Anat.*, 1932-33, **56**, 113-115.
16. Ahlbom, H. E. Mucous- and salivary-gland tumours. *Acta radiol.*, 1935, suppl. **23**, 1-452 (Table XV, Figures 29 and 30).
17. Lloyd, O. C. Salivary adenoma and adenolymphoma. *J. Path. & Bact.*, 1946, **58**, 699-710.
18. Godwin, J. T., and Colvin, S. H., Jr. Adenoma of the parotid gland. *Arch. Path.*, 1948, **46**, 187-189.
19. Bauer, W. H., and Bauer, J. D. Classification of glandular tumors of salivary glands. Study of 143 cases. *A. M. A. Arch. Path.*, 1953, **55**, 328-346.
20. Buxton, R. W., Maxwell, J. H., and French, A. J. Surgical treatment of epithelial tumors of the parotid gland. *Surg., Gynec. & Obst.*, 1953, **97**, 401-416.

## LEGENDS FOR FIGURES

FIG. 1. Gross photograph of a recurrent acinic cell tumor of the parotid gland demonstrating lobulations.  $\times 5$ .

FIG. 2. Recurrent acinic cell tumor showing lobulations with intervening fibrous stroma. Hematoxylin and eosin stain.  $\times 7\frac{1}{2}$ .





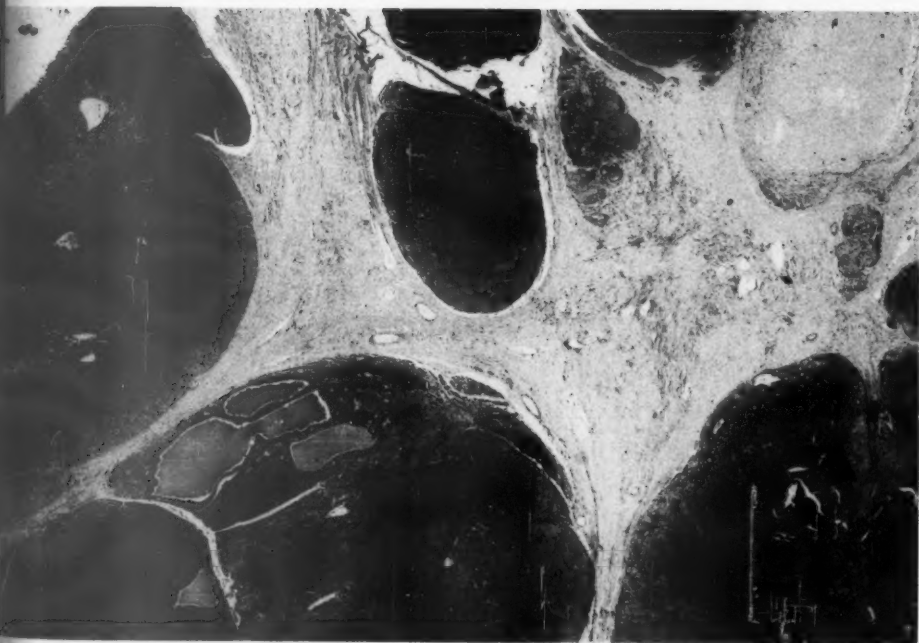
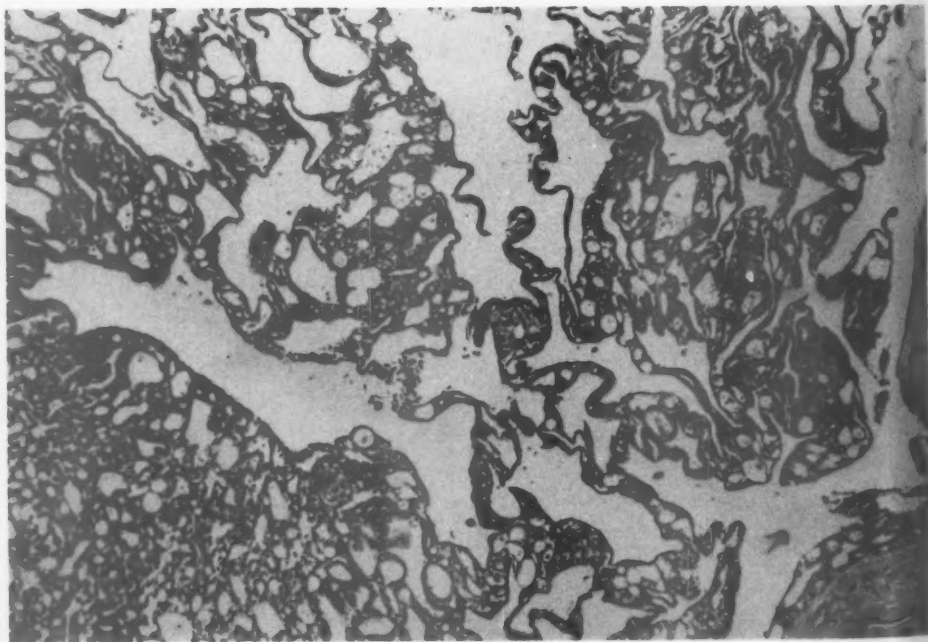


FIG. 3. Section of a papillary cystic pattern infrequently encountered. A higher magnification of this section revealed good cytoplasmic granularity. Hematoxylin and eosin stain.  $\times 50$ .

FIG. 4. Section showing a more glandular pattern and resembling the lesion reported by Masson<sup>10</sup> in 1924. Hematoxylin and eosin stain.  $\times 195$ .

FIG. 5. Section demonstrating little granularity of cytoplasm and prominent intercellular vacuolization. Hematoxylin and eosin stain.  $\times 195$ .





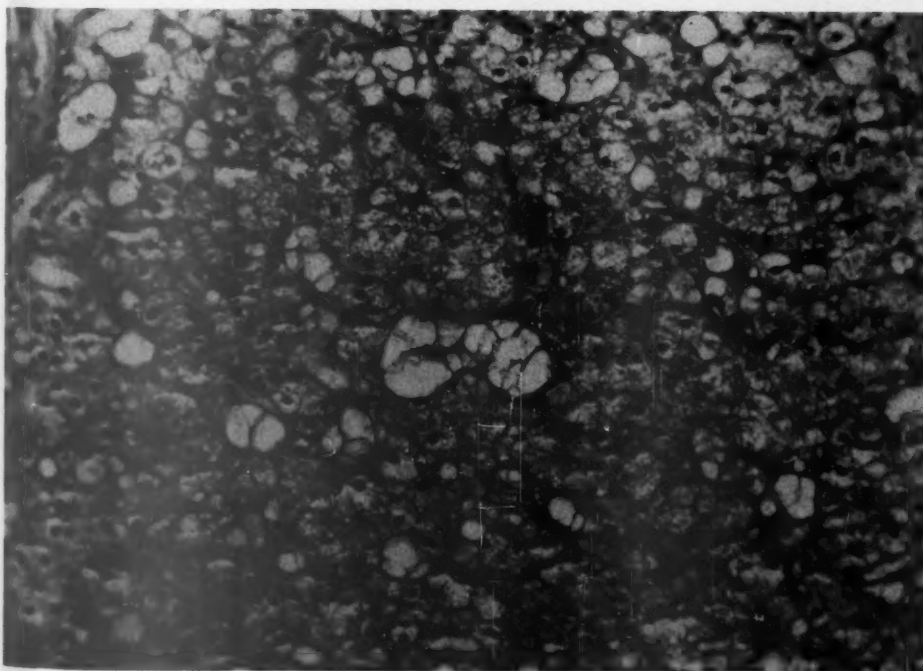
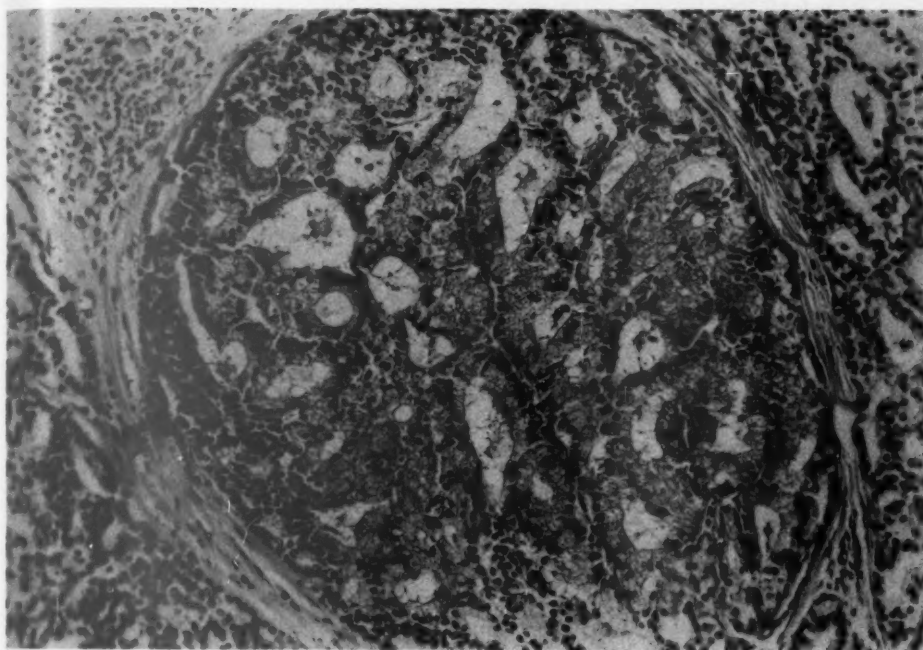


FIG. 6. Photograph of frozen section of tumor stained with polychrome methylene blue. For comparison with normal parotid gland in Figure 7.  $\times 210$ .

FIG. 7. Photograph of frozen section of normal parotid gland stained with polychrome methylene blue. For comparison with Figure 6 from an acinic cell tumor similarly stained.  $\times 195$ .

FIG. 8. Intracellular granules and intercellular spaces in an acinic cell neoplasm. Hematoxylin and eosin stain.  $\times 435$ .



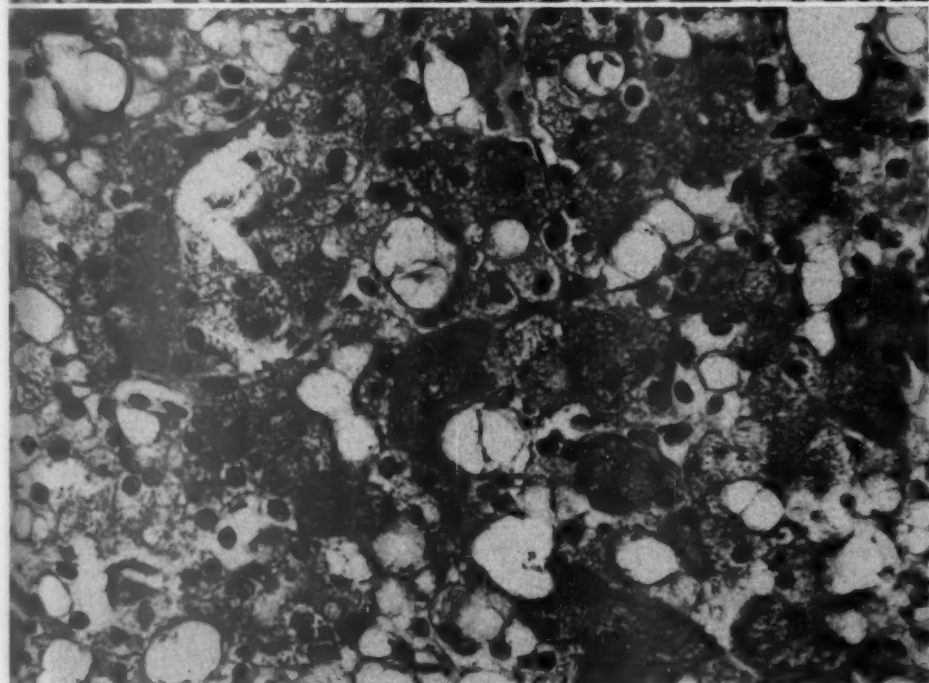
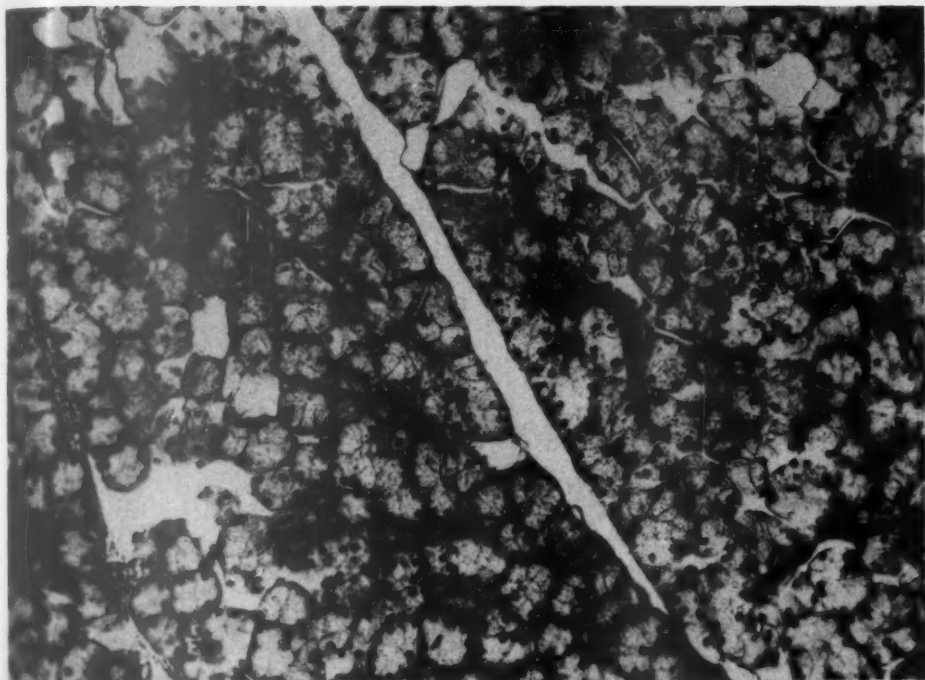
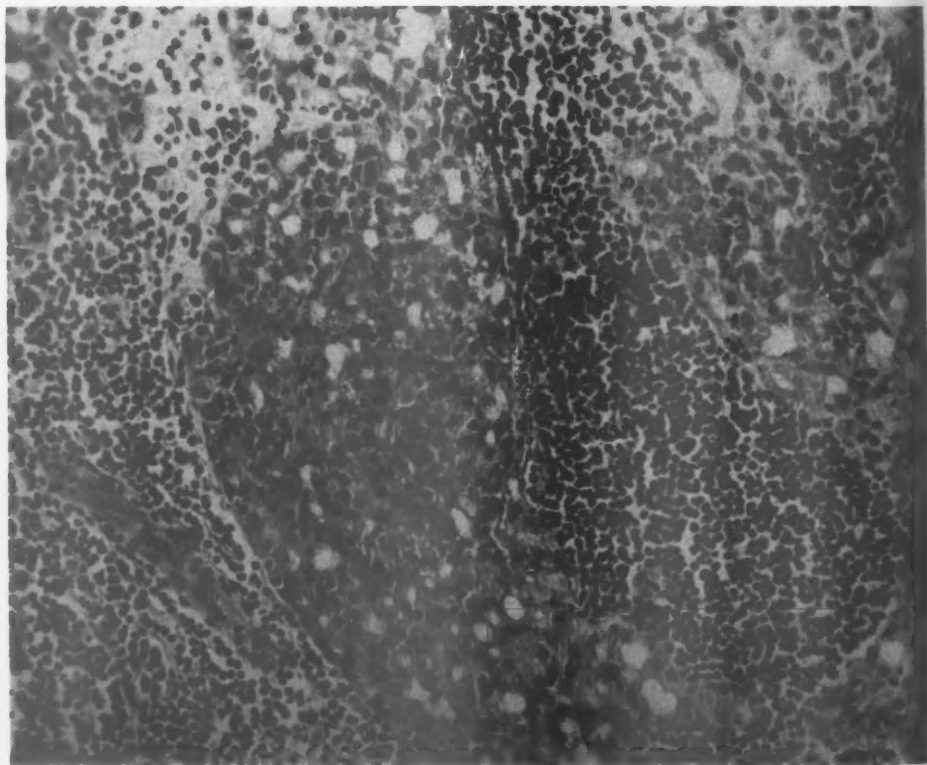
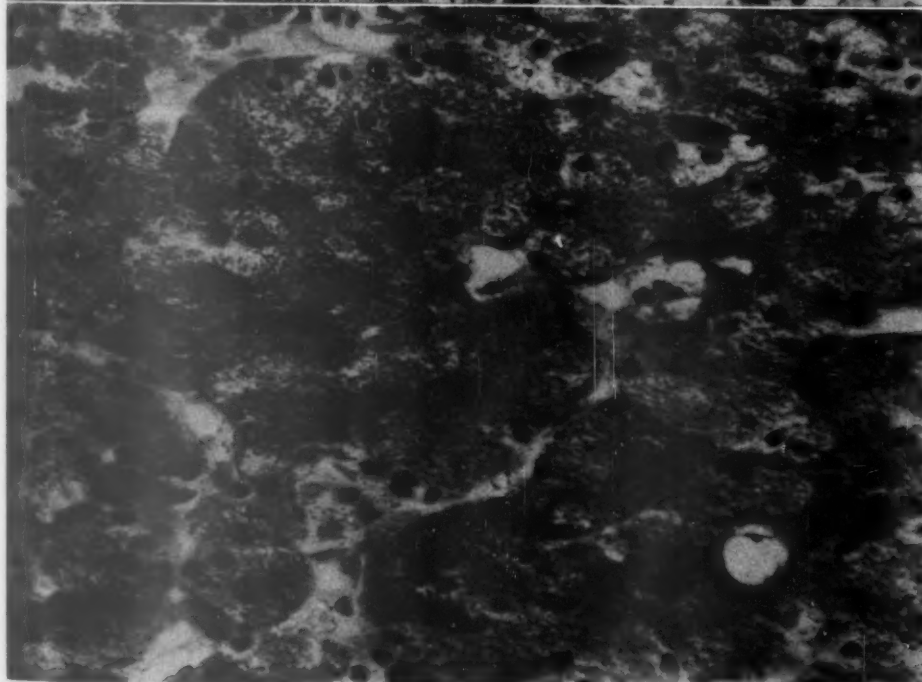
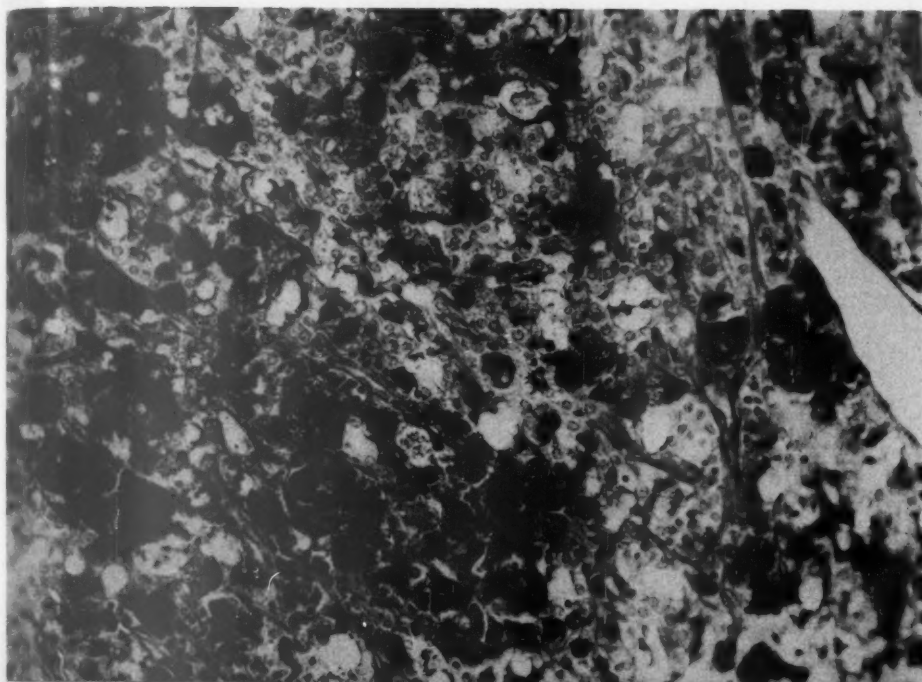


FIG. 9. Neoplasm with lymphoid stroma. Hematoxylin and eosin stain.  $\times 465$ .

FIG. 10. Periodic acid-Schiff's stain demonstrating positive-staining cells with intracellular granules, and intervening negatively stained cells. The normal parotid acinic cell also gives a positive stain.  $\times 210$ .

FIG. 11. Section of a pulmonary metastasis from case 27. A local recurrence showed similar granules. Hematoxylin and eosin stain.  $\times 465$ .









## INTERMEDIATE NEPHRON NEPHROSIS FROM SNAKE POISONING IN MAN

### HISTOPATHOLOGIC STUDY \*

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We recently had occasion to study at necropsy the lesions in individuals bitten by rattlesnakes of the species *Crotalus terrificus terrificus*. We were surprised that the renal lesions appeared similar to those in the so-called crush syndrome. Before this syndrome was described in English as crush syndrome and later as lower nephron nephrosis, it had previously been studied in Germany by Minami (1923), who designated it Nephrose nach Verschüttung or Verschüttungs-nephrose.

The pure acute renal inflammations, as observed during the first world war, were studied by Herxheimer, Dietrich, Gräff, and others. The essentially parenchymatous, and predominantly tubular changes of the kidney were described by Hackradt (1917) and by Groll (1921) on the basis of medical experience during the war of 1914-18. Hackradt established a very clear distinction between the actual war nephritis, represented by acute hemorrhagic glomerulonephritis with all the signs of inflammation, and the fatal acute vasomotor nephritis produced by burial under debris (Verschüttungs-nephrose). Borst (1917) characterized the acute nephrosis found after such crushing as of vasomotor origin. In similar cases, Bredauer (1920) reported anemic infarcts of the kidney, spleen, and liver which, according to Borst, might not have been caused by vascular lesions or thrombi, but by vascular spasms of traumatic origin.

Minami (1923) studied the material collected by Pick during the first world war and published his observations on the kidneys in 3 typical cases of crush nephrosis (Verschüttungs-nephrose). All 3 patients were apparently healthy individuals, who died between the fourth and seventh days after having been crushed. In his illustrations, Minami showed typical casts of the medullary zone, as well as infiltration of small round cells around the casts. The casts were irregular and of a more or less brownish color. They frequently were seen in the loops of Henle and, less frequently, in the convoluted tubules. In histologic sections, the benzidine reaction of Lepehne was always

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positive, producing a dark brown coloration. Yet, in the 3 cases studied both the reaction to iron and that to Sudan were negative. In the 3 cases observed, the microscopic examination disclosed an extremely important fact, namely, that [translated] "there was no deviation of the normal blood content, either in the glomeruli or in the thinnest renal vessels." The volume of the glomeruli was not enlarged and the capsular spaces contained finely granular deposits. In contrast, the urinary canaliculi and particularly the convoluted tubules showed remarkable changes, with the familiar picture of acute parenchymatous degeneration or of acute nephrosis. Dirty brown or grayish brown casts appeared frequently, especially in the collecting tubules, at the medullary radiations. These casts reacted negatively both to Sudan and for iron. They were positive when stained with Nile blue sulfate, with neutral red, and also with the Smith-Dietrich stain. Minami shared the opinion of Bredauer as to the pigment of the casts, which, in view of their morphologic and microchemical behavior, seemed to be composed of methemoglobin. According to Minami, nephrosis combined with more or less intense methemoglobin tubular plugging consequently existed in such cases. He confirmed Miller's concept, according to which only the epithelium of the proximal convoluted tubules and the epithelium of Henle's loop plays an active rôle in the elimination of hemoglobin. The absence of this substance in the glomeruli and in the capsular space was in favor of Miller's concept.

Minami discussed the rôle of various causal factors in hemoglobinemia and hemoglobinuria, including among others the toxic products of metabolism (autotoxins), cold, and toxic myogenic products of metabolism. These factors induce hemolysis and the formation of methemoglobin, which in turn would cause methemoglobinemia and methemoglobinuria. There would thus be a close relationship between these factors and the formation of hematic casts found in the kidney after crushing. From Minami's investigations we draw the conclusion that there would hence exist two forms of crush nephrosis: the traumatic-vasomotor form of Borst-Hackradt and the traumatic-toxic form of Pick-Minami.

Bywaters and Dible (1942) assembled in one report 22 cases of crush syndrome. Ten of these cases had already been studied by other authors. In all of them the urine was acid, red or dark, and gave a positive benzidine reaction. The spectroscopic examination disclosed the presence of myohemoglobin in the urine, which also contained albumin. An examination of the sediment showed casts and some erythrocytes. The patient was apparently well during treatment.

Later oliguria or anuria occurred. The blood showed an increase in the concentration of urea, phosphates, and potassium. By the end of the first week, the patient was either cured after diuresis or death occurred. Microscopic examination showed complete necrosis in small areas of the intermediate zone, seriously affecting the ascending limb of Henle's loop and the second distal convoluted tubule. There occurred rupture of the tubule and protrusion of the casts into the interstitial tissue, with surrounding histiocytic reaction and fibrosis in the more advanced lesions. Within the tubules, the casts had been invaded by polymorphonuclear neutrophilic leukocytes, the medullary zone showing a more pronounced invasion. "It may be so intense as to recall the picture of the pus-filled tubules of ascending pyelonephritis." Yet the inflammation seemed to be aseptic, without pyelitis. The casts appeared more typically and more frequently in the collecting tubules, particularly in transverse sections of the papillae. They were granular and homogeneous, with a positive benzidine reaction. The authors stated that: "We may assume that this intratubular pigment is myohaemoglobin or one of its derivatives."

Lucké (1946) undertook an extensive study of the question, based on 538 fatal cases during the second world war. The presence of "heme" casts and of degenerative lesions brought about the use of the name hemoglobinuric nephrosis, but the characteristic location of the lesions led the author to change this term to lower nephron nephrosis as being more appropriate ("the location of the lesions is so characteristic and unique, that the term 'lower nephron nephrosis' seems more descriptive and has been adopted"). The collecting tubules very seldom presented lesions of a marked degree, but contained casts of "heme." The stroma was edematous with foci of inflammatory reaction surrounding regions of tubular disintegration. At first the lymphocytes and histiocytes predominated. Granulocytes were rare and giant cells occurred occasionally. Later fibroblasts appeared and eventually the formation of scars occurred. Sometimes, when the tubules became necrotic, they burst into veins, producing thrombi, which were frequently parietal, rarely obstructive. The casts were more evident in the collecting tubules than in the lower portion of the nephron in spite of the fact that this region was seldom affected by degenerative changes. The specific renal lesions, essentially limited to the lower segments of the nephron, showed the following characteristics: "focal degeneration or necrosis, presence of heme casts, secondary inflammatory reactions in the surrounding stroma, and thrombosis of thin walled veins."

In an extensive study, Pizarro (1950) assembled 24 morbid condi-

tions capable of developing nephrosis of the lower nephron. He made a special study of the frequency of the syndrome in obstetric cases. More recently, Meessen (1952) discussed an identical nephrosis in the rabbit brought about by the subcutaneous injection of a 2 per cent solution of uranium ("Uranephrose"). Elective lesions of the cortical-medullary boundary zone appeared after 5 days. In injected pregnant rabbits, uranium produced nephrosis in the fetus, but of lesser intensity than in the maternal kidney.

It is interesting that poisoning through snake bite is not found among the multiple causal agents pointed out as apt to cause this syndrome. As a matter of fact and as emphasized in our previous paper (Amorim, Mello, and Saliba, 1951), there are very few publications on the pathologic anatomy of snake poisoning both with regard to experimental investigation on animals and to the study of necropsied human cases of poisoning from snake bite. As to the human pathologic anatomy, the few publications found refer only to necropsies on individuals bitten by snakes of the genus *Bothrops*, as stated in a previous paper. With regard to poisoning through snake venom of the genus *Crotalus*, the publications found in the literature are all of an experimental character, such as the papers of Mitchell (1860, 1868), Mitchell and Reichert (1886), Pearce (1909), Taube and Essex (1937), and Fidler, Glasgow, and Carmichael (1940). Furthermore, these publications refer to species existing in North America—*Crotalus adamanteus*, *Crotalus atrox*, and others non-existent in South America, where only *Crotalus terrificus terrificus* (Laurentius, 1768) is found.

Besides representing a new contribution to the study of the lesions produced in man by the venom of the rattlesnake (*Crotalus terrificus terrificus*), our observations point out a new factor in the development of hemoglobinuric nephrosis.

#### OBSERVATIONS AND DESCRIPTION OF THE LESIONS

We had an opportunity to study 3 cases of rattlesnake bite in white males, the first 58, the second 30, and the third 12 years of age. In the first and second cases, the men were bitten in the foot by rattlesnakes while working in the fields. The first symptoms were visual disturbances and diminished eyesight, with dizziness and pain.

##### Case 1

P. J. da C. was 58 years old. In the evening following snakebite, the urine already appeared the color of blood. Three days later the patient presented himself for consultation at the Butantan Institute, showing prostration and haggard facies. Temperature was 36.9° C.; pulse, 110; blood pressure, 130/80 mm. of Hg. On the

following day the temperature went down to 36.1°; pulse, 120; blood pressure, 130/90. Anuria developed. The patient was removed to the hospital and treated by the artificial kidney. He died 5 days after the accident. From the first day on, he had received subcutaneous injections of anticrotalic serum totaling 160 cc.

### Case 2

B. G., 30 years old, was admitted to the emergency ward of the Butantan Institute on the day that he was bitten, complaining of loss of eyesight and dizziness. Temperature was 36.5° C.; pulse, 72; blood pressure, 140/90; clotting time, 5 minutes; bleeding time, 1½ minutes; urine appeared bloody and wine colored. In spite of coadjuvant medication, the blood pressure fell on the first day. One day later there was ptosis of the eyelids and improvement of the general condition. Urine was scant and wine colored. Blood pressure rose again to 130/90. Two days later, the patient showed an improvement of his general condition. He spoke with more ease, reported a sensation of well-being, moved more, but was nauseated and had difficulty in urinating (290 cc. in 24 hours, always of wine color). Blood pressure was 140/90. There was intense hemoglobinuria. Death occurred 3 days after the accident.

### Case 3

J. M. A., 12 years of age, was examined at the Butantan Institute on the same day that he was bitten. He showed uremia, acidosis, albuminuria, hematuria, and hemoglobinuria. At first there was oliguria, then anuria, the patient dying in uremia 7 days after the accident.

### MACROSCOPIC EXAMINATION

Omitting the secondary lesions without immediate relation to our problem, the necropsy of case 1 disclosed the following: The *brain* was edematous. There were small hemorrhagic suffusions in the leptomeninges, more intense in the right temporal and parietal lobes. Extensive hemorrhages had occurred in the posterior poles of the cerebellar hemispheres, chiefly on the left side. There was blood in the frontal poles of the lateral ventricles. *Kidneys* (Fig. 1) were increased in volume, the capsule easily detachable leaving a smooth surface. External and sectioned surfaces were of an opaque pale pink, having the appearance of cooked meat. Consistency was firm. The *bladder* was distended, containing 150 cc. of urine of a reddish color, with hyperemia and dilatation of the vessels of the mucosa. Case 2 showed nothing noteworthy macroscopically. Case 3 (Fig. 2) showed hyperemia of the brain and of the leptomeninges, lungs, suprarenal glands, and liver, but no hemorrhages. The liver was fatty.

### HISTOPATHOLOGIC EXAMINATION

In order to avoid repetition, we shall limit the histopathologic report to the first typical case, referring only to the differences which appeared among the three cases, since the principal lesions were fundamentally alike.

### *Kidney*

Various diffuse hemorrhagic foci were present in the fat tissue of the renal hilus. Intense hyperemia was present in the capillaries, which showed conglutination of the erythrocytes due to stasis.

### *Cortex*

Intense hyperemia was noted in several cortical areas, with maximal vasodilation of the precapillaries. These presented lumina filled with erythrocytes, but their boundaries were distinct and there was no conglutination of the blood elements. There was consequently no actual stasis, but merely stagnation of the blood. Nearly all capillaries of the cortical zone showed this condition of intense hyperemia, including those in the renal glomeruli. In various parts of the cortex were dilated tubules with lumina full of desquamated cells and neutrophilic granulocytes. In some of these the wall was poorly outlined, surrounded by clearly defined histiocytic proliferation and by marked interstitial infiltration by neutrophilic granulocytes. Such inflammatory cortical areas showed a focal distribution identical to that of the degenerative lesions and the distribution of hyaline casts to be described. Sometimes there was noticeable in the central part of such foci, under slight enlargement, one or more sections of tubules containing eosinophilic hyaline casts and, surrounding them, various sections of dilated tubules containing reddish granular or fragmented material mixed with desquamated cells and neutrophilic granulocytes.

*Glomeruli (Fig. 3).* Of the enlarged glomerular loops, some were empty and others filled with erythrocytes, with one or the other of these appearances predominating. Neither neutrophilic leukocytes nor an increase of nuclei was seen. The capsule was very thin, with simple desquamation of the lining cells. The capsular space was filled with a delicately pink, granular or reticulate substance. Glomerular afferent arterioles showed nothing except occasional slight enlargement.

*Tubules.* The proximal convoluted tubules showed intense cloudy swelling with abundant granulo-albuminous content in the lumina. The brush border was preserved in the majority of cells, but in some it was not visible. In certain tubules some cells were in mitosis, showing numerous chromosomes irregularly arranged in the equatorial plane, some round and others elongated. They sometimes formed an asymmetric equatorial plate with various aberrant chromosomes located on the outside of the achromatic spindle. In one cell the appearance was that of an atypical polyploid mitosis (Fig. 12). The ascending limbs of Henle's loop and the distal convoluted tubules



frequently appeared enlarged, with flattened epithelium and lumina filled with deposits. Some of these were granular (Figs. 5 and 6) and others of hyaline aspect surrounded by detached cells mixed with neutrophilic leukocytes. Other distal tubules showed swollen cells, with cytoplasm rich in hyaline eosinophilic granulations. Some cells had disappeared and others showed pyknotic nuclei (Fig. 4). The lesions were distributed in foci. Near the glomeruli the tubules frequently appeared enlarged to the maximum, the lumen being filled chiefly with detached cells and neutrophilic leukocytes, resembling a catarrhal-desquamative lesion. Sometimes at a greater distance from the glomeruli the lumen contained only masses of hyalinized, strongly eosinophilic substance (Figs. 3 and 11).

#### Intermediate Zone

The greatly enlarged ascending limbs of Henle's loop presented lumina filled with casts of hyaline material, intensely stained by eosin (Figs. 7 and 8). The casts sometimes formed a homogeneous block and sometimes were composed of an agglomeration of numerous fragments of homogeneous structure. The cells of the tubules usually were cubical, in some regions they were flattened, and in certain parts they were either desquamated and present or were absent. The nuclei showed pyknosis and karyolysis. In the intermediate zone next to the medulla only casts were found, without interstitial inflammatory changes (Fig. 13). However, nearer the cortex, many tubules contained neutrophilic leukocytes (Fig. 13) and the interstitial connective tissue presented intense inflammatory infiltration, chiefly by neutrophilic leukocytes, with marked edema and dissociation (Fig. 8). At the same time, in these regions an intense desquamation of the epithelial cells was noticed in several tubules, even in those in which no casts or hyaline material were found. Several areas also showed strong histiocytic proliferation in the stroma (Fig. 9), beside infiltrations by lymphocytes and some plasma cells. Thus, in the intermediate zone, the inflammatory process was rather intense, as indicated by the heavy migration of neutrophilic leukocytes inside the tubules and by the inflammatory reactions with edema, neutrophilic granulocytes, and histiocytic proliferation in the interstitial tissue.

#### Medulla

The medullary zone (Fig. 14) presented a characteristic appearance because of the presence in the interior of many of the collecting tubules of Bellini (but not in all of them) of either hyaline casts or of casts

showing granular structure and containing desquamated cells and neutrophilic leukocytes. However, the interstitial tissue around and between the tubules appeared absolutely free from inflammatory changes. This aspect stood in impressive contrast to the intense inflammatory reaction in the intermediate zone and also in foci in the cortex as previously described (Figs. 8, 9, 11, and 13). Some of the tubules of Bellini presented the epithelial cells as desquamated only, without any trace in their interior of the presence of casts. By the Lepehne method with benzidine, the casts contained in the tubules, both in the medulla and in the intermediate zone, and even some in the cortex, reacted positively for hemoglobin, showing a dark brown color (Fig. 10). Consequently, we wish to emphasize the complete absence of inflammation in the medulla, which appeared "clean" in the sections, merely showing the typical casts of hemoglobin inside the tubules (Figs. 10 and 14). On the other hand, and in contrast to this, was the marked interstitial inflammatory reaction (Figs. 8 and 9), in addition to the severe degenerative changes in the tubules and the hyaline casts, in nearly the whole intermediate zone and distributed in foci in the cortex reaching up to the cortex corticis.

In case 2 only hyperemia of the glomeruli but no inflammatory phenomena were observed (Figs. 5 and 6). Case 3 showed evident glomerular ischemia, the loops appearing enlarged, or as if they were inflated. There was, however, an increase in neutrophilic granulocytes in the glomeruli. No hyperemia in the capillaries of the labyrinthine zone was noted. Obvious foci of interstitial inflammatory reaction surrounded by hyperemic-hemorrhagic areas existed in the boundary zone. In some regions, the inflammatory reaction consisted of a discrete infiltration of neutrophilic leukocytes with a more intense proliferation of histiocytic macrophages. These sometimes formed dense clusters or small nodules between the tubules, which were either comparatively well preserved or in necrobiosis. The appearance of these foci was granulomatous, some of these small isolated histiocytic nodules suggesting Aschoff nodes.

Therefore, we are here dealing with a *nephrosis which is predominant in the intermediate or boundary zone* of the kidney with secondary hyperemic inflammatory reaction in foci, the appearance of which is sometimes granulomatous.

#### SUMMARY OF OBSERVATIONS

The three cases examined presented a syndrome characterized by severe and progressive anuria, bloody, wine-colored urine, hemoglo-



binuria with visual disturbances and other neurologic phenomena, death occurring within a few days (3 to 5) after the accident. Necropsy showed that the principal lesions appeared almost exclusively in the kidney, characterized by severe necrobiotic degeneration predominantly in the ascending limb of Henle's loop and in the so-called "intermediate segment" of Schweigger-Seidel of the distal convoluted tubules. There we observed granular and hyaline degeneration of the cells, pyknosis, karyolysis, vacuolar degeneration (Fig. 6), steatosis, and also the disappearance of many of these cells. In the lumen of the tubules hyaline casts were seen, sometimes formed by a single mass, at other times by irregular or globular fragments of a brownish or reddish brown color in sections stained by hematoxylin and eosin (Figs. 7, 8, and 11). These reacted typically to benzidine by the Lepehne method (Fig. 10), thus demonstrating their hemoglobinic nature.

In the medulla the cells of the collecting tubules were uninjured, particularly in the first and third cases. The tubules contained the typical hyaline hemoglobinuric casts in large numbers (Figs. 10 and 14). In the intermediate zone (boundary zone) there were more severe degenerative or parenchymatous lesions, and a strong inflammatory reaction of the interstitial tissue with edema and migration of neutrophilic leukocytes, lymphocytes, and plasma cells. There was also a striking histiocytic reaction in certain areas (Figs. 8, 9, and 13). Many casts were invaded by neutrophilic leukocytes (Fig. 13). These inflammatory lesions were reactionary or secondary to the destruction of the epithelium and matched the degenerative lesions in their intensity. Thus they were more noticeable in the cortex and in its boundary zone around the ascending limbs of the loops and distal convoluted tubules where the degenerative lesions were more severe. Only casts were found in the medulla and it was free from inflammatory phenomena (Fig. 14) because there were no degenerative lesions of the tubular cells. Thus, the medulla represents but an indicator, a place for the ready encounter of hemoglobinuric casts, which are pathognomonic of the lesional syndrome.

#### DISCUSSION

The lesions found by us coincide exactly with those reported by Minami, Hackradt, and Bredauer under the name of *Verschüttungs-nephrose* and by Bywaters and Dible, Lucké, and others under the term *crush syndrome*. In view of the great importance of these lesions, we will discuss some questions related to the interpretation of our ob-

servations, principally those with regard to the concept of nephrosis, and we should also like to attempt to establish a more adequate nomenclature for the lesions we encountered.

*Lesions Produced by Crotalic Venom in the Light of the New  
Concepts of Nephrosis*

Lemos Torres and Lemos Torres, as early as 1940, called attention to the doubts existing as to the concept of nephrosis, emphasizing that the nosographic entities are abstract notions, resulting from a schematic generalization imposed by mere necessity of classification. These authors then brought up the question whether one should reject nephrosis as an autonomous clinical entity. They cited the well known work of Randerath (1937), who defined nephrosis as primarily a modification of the permeability of the glomerular capillary, which may or may not be accompanied by lesions of the capillary wall, and which secondarily may produce morphologic changes in the epithelium of the tubules and of the glomeruli. Fahr (1938) and other authors objected to this concept, insisting upon the division of nephrosis into glomerulonephrosis and tubulonephrosis.

Ellis (1942) undertook a study reviewing the concept of nephrosis. He investigated 600 cases of Bright's disease which had been observed clinically. Reports had been made on necropsies and histologic examinations of 200 of these cases. He then made the following statement: "I do not think that this conception of nephrosis as a separate entity is justified." He found in cases clinically regarded as nephrosis the histologic changes of nephritis of type 2 and, in other cases appearing clinically as nephritis of type 2, the histologic picture of nephrosis, as revealed by necropsy. In this sense, according to Ellis, the essential differentiation should not be made between nephrosis and nephritis, but primarily between nephritis of type 1 and nephritis of type 2. Also, many cases classified as nephrosis on a clinical and histologic basis would be included in the series of nephritis of type 2.

Hamburger (1948 and 1950) believed that the term nephrosis ought not to be used, because the process is as much inflammatory as degenerative, deserving the name of nephritis. He stressed the fact that besides the prominent tubular changes there appear foci of necrosis, and also a reaction of the interstitial tissue with lymphoplasmocytic infiltrates, side by side with perivascular inflammatory lesions showing a certain degree of fibroblastic proliferation, and with intravascular thrombosis. The tubular lesions extend along the whole length of the tube, affecting both the proximal portion and the loop of Henle, in-

cluding the distal tube. Hence, according to Hamburger, the employment of the name lower nephron nephrosis, as proposed by Lucké (1946), would be inadequate. He believed that the more correct name would be tubulonephritis, placing this disease group in opposition to the glomerular nephropathies.

The renal lesions caused by crotalic venom seem to be primarily of the renal tubule and of a degenerative parenchymatous character. Secondly, inflammatory reactions then appear, which thus show a reactional or symptomatic character such as is the case with certain lesions of the nervous centers (concept of symptomatic inflammation according to Spielmeyer, 1922). Therefore, we note that the lesions primarily show a predominant nephrotic picture and, in second place, reactionary inflammatory phenomena (moderate nephritis).

*Lower Nephron Nephrosis or Intermediate Nephron Nephrosis?*

Poirier and Charpy (1923) divided the uriniferous tubule after the glomerulus into three segments: labyrinthine segment (tubulus contortus), medullary segment (Henle's loop), labyrinthine segment (intermediate canal), after which follows the series of medullary segments (collecting tubules). Peter (1909), through the method of dissociation and of reconstructions, discerned six segments, the last of which (F, intermediate canal or distal convoluted tubule) comprised a thick, dark, meandering portion between two finer and clearer portions. Policard (1908 and 1950) assigned the name intermediate segment to those parts, corresponding to segments D, E, and F of Peter, which are characterized by the possession of an epithelium with basal rods, without cuticle and without neatly outlined intercellular limits. This segment without striated cuticle (segment III) includes the ascending branch of the loop of Henle and the intermediate canal (F) of Peter.

Hovelacque and Turchini (1938) called the second, 6 mm. long, convoluted segment, the intermediate piece or segment of Schweigger-Seidel (1865), interstitial segment, or distal segment (tubulus contortus II, pars convoluta II). However, they preferred the classical division of Schweigger-Seidel as being "la plus généralement adoptée."

Braus (1924) designated the first segments of the uriniferous tubule as the nephron, as these form the secretory part of the kidney. The last segment would be the excretory part. Da Costa and Chaves (1944) referred to the second widened cortical portion of the ascending branch of the loop as the intermediate piece of Schweigger-Seidel, distal segment of the uriniferous tubule or tubulus contortus II. They

confirmed the concept of Braus, adding that the stretch of the uriniferous tubule, beginning with the enlargement which involves the glomerulus (Bowman's capsule) up to the intermediate piece of Schweigger-Seidel, is related to the formation of urine, constituting the actual "nephronium."

Without mentioning the numerous synonyms in existence for the various parts of the uriniferous tubule nor the reason why he simplified this nomenclature, Lucké (1946) made his division into three segments: proximal (upper), intermediate, and lower. The intermediate segment would be the narrow portion of Henle's loop. The lower segment would be formed by the wide part of Henle's loop and by the distal convoluted tubule. Lucké regarded these two portions as a histologic and functional unit—the lower segment.

In spite of all this, we think that from the point of view of pathology, the real distal or lower segment of the renal tubule is located in the medullary zone or in the collecting tubules up to the renal papilla. There are pathologic renal pictures located only in the medullary zone or in the collecting tubules, such as, for instance, the lesions produced by uric acid gout and experimental poisoning provoked by various chemical substances (vinylamin, tetra-hydroquinoline, bromethyl-amino-bromhydrate, and others) recently mentioned by White and Mori-Chavez (1952). For this reason we should like to suggest that it would perhaps be more convenient and in accordance with the existence of several lesional pictures in renal pathology to include under the heading of nephron not only the secretory part of the uriniferous tubule, which would comprise the intermediate nephron, but also the excretory part with inclusion of the collecting tubule, which would then be the real lower nephron. It is obvious that such a suggestion would imply a broadening of the concept of nephron, but would not fail to offer certain advantages. Thus, the category of intermediate nephron nephrosis would state with greater precision the localization of the lesions in this part of the uriniferous tubule. The designation of lower nephron nephrosis would then be reserved for the lesions predominating in the excretory part of the uriniferous tubule, which includes the whole medullary portion of the kidney where pure parenchymatous lesions have been described. This would also actually be real nephrosis.

The lesions produced by the venom of *Crotalus terrificus terrificus* predominate in the intermediate nephron (Figs. 3, 4, 8, 9, and 13), thus provoking an intermediate nephron nephrosis according to our classification. The lesions found in the lower nephron (Fig. 14) thus

defined would be of secondary order, with the presence of hemoglobin casts which would have developed previously in the intermediate nephron.

#### SUMMARY AND CONCLUSIONS

A report is made of the histopathologic findings, with a detailed study of the renal lesions, in three human patients bitten by rattlesnakes of the species *Crotalus terrificus terrificus*.

The renal lesions consisted particularly of degenerative and necrotizing processes, especially in the ascending portion of Henle's loop and in the distal convoluted tubules, in which casts were noted. These reacted positively to hemoglobin by the benzidine method of Lepehne. They were numerous also in the medullary zone, within the collecting tubules of Bellini. Inflammatory interstitial reaction with neutrophilic leukocytes and edema, and occasional marked focal hyperplasia of histiocytes appeared in the intermediate zone and in the cortex around the degenerated tubules. In contrast, attention is called to the fact that inside the tubules of Bellini only casts without degenerative or necrotic changes appeared, and consequently without inflammatory reaction. This feature, especially emphasized by us, characterizes the medullary zone as "clean," in contrast to the intermediate and cortical zones of the parenchyma, where the histologic image is less clear because of the presence of reactionary inflammatory lesions.

Such histopathologic findings correspond exactly to the lesions described by Minami, Hackradt, and Bredauer under the designation of Verschüttungs-nephrose, and by Bywaters and Dible, and others under the name of crush syndrome. Later, Lucké assigned to this same syndrome the name lower nephron nephrosis, which is usually characterized by hemoglobinuria and hemolytic anemia. Until now, these lesions have not been reported in cases of poisoning by ophidian venom (*Crotalus terrificus terrificus*), which consequently means that a new factor has to be added to the causes which produce hemoglobinuric nephrosis.

The significance of the lesions caused by the crotalic venom is discussed in the light of the new concepts of nephrosis which tend to assign greater importance to the inflammatory process than to the degenerative changes in identical lesional pictures. The lesions produced by the venom of the rattlesnake (*Crotalus terrificus terrificus*) primarily showed a degenerative process (nephrosis) and secondarily an inflammatory reaction (moderate nephritis), recalling certain lesions of the nervous centers (Spielmeyer's concept of symptomatic inflammation).



The problem is also raised of the nomenclature considered most adequate for these lesions. It is known that in hemoglobinuric nephrosis the parts of the uriniferous tubule most involved are those termed, within the existent synonymy, intermediate segment by the majority of authors since Schweigger-Seidel. Moreover, the principal lesions are more intense and marked in the boundary or intermediate zone and in the renal cortex and less so in the medulla. In view of this, we suggest that this lesion be called intermediate nephron nephrosis or necrotizing nephrosis of the intermediate nephron, as opposed to Lucké, who named it lower nephron nephrosis.

It would seem more advisable to reserve the term lower nephron nephrosis in renal pathology for such lesions as predominate in the distal portion of the uriniferous tubule. Actually, the real distal or lower segment of the renal tubule is located in the medulla (collecting tubules) up to the renal papilla, where some degenerative or necrotizing processes take place, such as, for instance, those caused by uric acid gout and by several chemical substances recently enumerated by White and Mori-Chavez (1952). These would then be the real lower nephron nephrosis.

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#### REFERENCES

- Amorim, M. F., Mello, R. F., and Saliba, F. Envenenamento Botrópico e Crotálico. Contribuição para o estudo experimental comparado das lesões. *Mem. Inst. Butantan*, 1951, 23, 63-108.
- Borst, M. Einwirkung der Schussverwundung und sonstiger Kriegsbeschädigungen auf die einzelnen Körpergewebe. In: Borchard, A., and Schmieden, V. *Lehrbuch der Kriegs-Chirurgie*. J. A. Barth, Leipzig, 1917, pp. 61-100. Also: *Pathologisch-anatomische Erfahrungen über Kriegsverletzungen. Samml. klin. Vorträge*, 1917, No. 735 (Chir. No. 201), pp. 297-328. (Cited by Minami, 1923.)
- Borst, M. Über Entzündung und Reizung. *Beitr. z. path. Anat. u. z. allg. Path.*, 1916-17, 63, 725-754. (Cited by Fahr in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*. Julius Springer, Berlin, 1925, 6, Pt. 1.)
- Braus, H. *Anatomie des Menschen; ein Lehrbuch für Studierende und Ärzte*. J. Springer, Berlin, 1921-24, 2, 351.
- Bredauer, K. *Pathologische Befunde bei Verschüttung im Kriege*. Inaugural Dissertation, München, 1920. (Cited by Minami, 1923.)
- Bywaters, E. G. L., and Dible, J. H. The renal lesion in traumatic anuria. *J. Path. & Bact.*, 1942, 54, 111-120.
- da Costa, A. C., and Chaves, P. R. *Tratado elementar de histologia e anatomia microscópica*. Livraria Luso-Espanhola Ltda., Lisbon, 1944, ed. 3, 2, 298.

- Dietrich, A. Ueberraschende Todesfälle durch Nephritis. *Berl. klin. Wchnschr.*, 1917, 54, 521-523. (Cited by Fahr in Henke-Lubarsch Handbuch, vol. 6, pt. 1.)
- Dietrich, A. Patologia General y Anatomia Patológica. F. Seix (ed.), Barcelona, 1941-43, 1, 256.
- Ellis, A. Natural history of Bright's disease; clinical, histological and experimental observations. *Lancet*, 1942, 1, 1-7; 34-36; 72-76.
- Fahr, T. Zur Frage der Nephrose. *Ztschr. f. klin. Med.*, 1938, 134, 533-562.
- Fidler, H. K., Glasgow, R. D., and Carmichael, E. B. Pathological changes produced by the subcutaneous injection of rattlesnake (*Crotalus*) venom into *Macaca mulatta* monkeys. *Am. J. Path.*, 1940, 16, 355-364.
- Gräff, S. Untersuchungen über das Verhalten der Leukozyten im Glomerulusgebiet bei der akuten Glomerulonephritis. *Deutsche med. Wchnschr.*, 1916, 42, 1092-1093. (Cited by Fahr in Henke-Lubarsch Handbuch, vol. 6, pt. 1.)
- Groll, H. Direkte Kriegserkrankungen durch größere physikalische Einwirkungen. In: von Schjerning, O. (ed.) Handbuch der Ärztlichen Erfahrungen im Weltkriege. J. A. Barth, Leipzig, 1921, 8, 506-513.
- Hackradt, A. Über akute, tödliche, vasomotorische Nephrosen nach Verschüttung. Inaugural Dissertation, München, 1917. (Cited by Minami, 1923.)
- Hamburger, J. Acquisitions récentes sur les maladies du rein en les acquisitions médicales récentes. Edit. Med. Flammarion, Paris, 1948.
- Hamburger, J. Acquisitions récentes sur les néphrites aiguës toxiques en les acquisitions médicales récentes. Edit. Med. Flammarion, Paris, 1950.
- Herxheimer, G. Nierenstudien. II. Über Anfangsstadien der Glomerulonephritis. *Beitr. z. path. Anat. u. z. allg. Path.*, 1918, 64, 454-476. (Cited by Fahr in Henke-Lubarsch Handbuch, vol. 6, pt. 1.)
- Herxheimer, G. Ueber das pathologisch-anatomische Bild der "Kriegsnephritis." *Deutsche med. Wchnschr.*, 1916, 42, 1969-1971. (Cited by Fahr in Henke-Lubarsch Handbuch, vol. 6, pt. 1.)
- Hovelacque, A., and Turchini, J. Anatomie et histologie de l'appareil urinaire et de l'appareil génital de l'homme. G. Doin & Cie., Paris, 1938, 306 pp.
- Laurentius, J. N. Specimen Medicum, exhibens Synopsis Reptilium Emendatum cum Experimentis circa Venena et Antidota Reptilium Austriacorum. Typ. Joan. Thom. Nob. de Trattnern, Vienna, 1768, pp. 1-214.
- Lemos Torres, A., and Lemos Torres, U. Sobre o conceito de nefroses. *Arq. de biol.*, 1940, 24, 156-163.
- Lepehne, G. Zerfall der roten Blutkörperchen beim Ikterus infectiosus (Weil). Ein weiterer Beitrag zur Frage des hämatogenen Ikterus, des Hämoglobins und Eisenstoffwechsels. *Beitr. z. path. Anat. u. z. allg. Path.*, 1919, 65, 163-226.
- Lucké, B. Lower nephron nephrosis (the renal lesions of the crush syndrome, of burns, transfusions, and other conditions affecting the lower segments of the nephrons). *Mil. Surgeon*, 1946, 99, 371-396.
- Meessen, H. Experimentelle Histopathologie. G. Thieme, Stuttgart, 1952, 153 pp.
- Miller, J. W. Über elektive Hämoglobinfärbung und der Ort der Hämoglobinausscheidung in der Niere. *Frankfurt. Ztschr. f. Path.*, 1912, 11, 403-422. (Cited by Minami, 1923.)
- Minami, S. Über Nierenveränderungen nach Verschüttung. *Virchows Arch. f. path. Anat.*, 1923, 245, 247-267.
- Mitchell, S. W. Researches upon the venom of the rattlesnake: with an investigation of the anatomy and physiology of the organs concerned. *Smithsonian Contrib. Knowl.*, 1860, 12, 1-145.



- Mitchell, S. W. Experimental contributions to the toxicology of rattle-snake venom. *New York M. J.*, 1868, 6, 289-322.
- Mitchell, S. W., and Reichert, E. T. Researches upon the venoms of poisonous serpents. *Smithsonian Contrib. Knowl.*, 1886, 26, 1-186. (Cited by Pearce, 1909.)
- Pearce, R. M. An experimental glomerular lesion caused by venom (*Crotalus adamanteus*). *J. Exper. Med.*, 1909, 11, 532-540.
- Peter, K. Untersuchungen über Bau und Entwicklung der Niere. G. Fischer, Jena, 1909, 446 pp. (Cited by Poirier and Charpy, 1923.)
- Pick, L. Zur pathologischen Anatomie der Verschüttungen. *Aerztl. Sachverst.-Ztg.*, 1920, No. 2. (Cited by Minami, 1923.)
- Pizarro, J. J. Síndrome de nefrose do nefron inferior em obstetricia. *Rev. ginec. obst.*, 1950, 2, 743-766.
- Poirier, P., and Charpy, A. *Traité d'anatomie humaine*. Masson & Cie., Paris, 1923, 5.
- Policard, A. *Précis d'histologie physiologique*. G. Doin & Cie., Paris, 1950, ed. 5, 858 pp.
- Policard, A. Le tube urinaire des mammifères. Masson & Cie., Paris, 1908, pp. 307-568.
- Randerath, E. Die Entwicklung der Lehre von den Nephrosen in der pathologischen Anatomie. *Ergebn. d. allg. Path. u. path. Anat.*, 1937, 32, 91-140.
- Schweigger-Seidel, F. Bemerkungen zu einer Arbeit über die "Harn- und Blutwege der Säugethier-Niere." *Wüzb. med. Ztschr.*, 1865, 6, 151-155.
- Spielmeyer, W. *Histopathologie des Nervensystems*. J. Springer, Berlin, 1922, 1, 426.
- Taube, H. N., and Essex, H. E. Pathologic changes in the tissues of the dog following injections of rattlesnake venom. *Arch. Path.*, 1937, 24, 43-51.
- White, J., and Mori-Chavez, P. Acute necrotizing renal papillitis experimentally produced in rats fed mono-N-methylaniline. *J. Nat. Cancer Inst.*, 1951-52, 12, 777-787.

#### LEGENDS FOR FIGURES

FIG. 1. Case 1. Cut surface of kidney showing dark coloration of the cortex. Gross photograph.  $\times 1\frac{1}{4}$ .

FIG. 2. Kidney of case 3 showing pale cortex and dark brown medulla. Boy 12 years old. Duration of illness, 8 days. Gross photograph. Natural size.





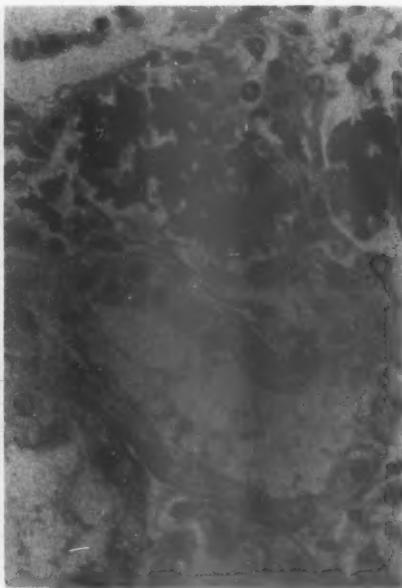
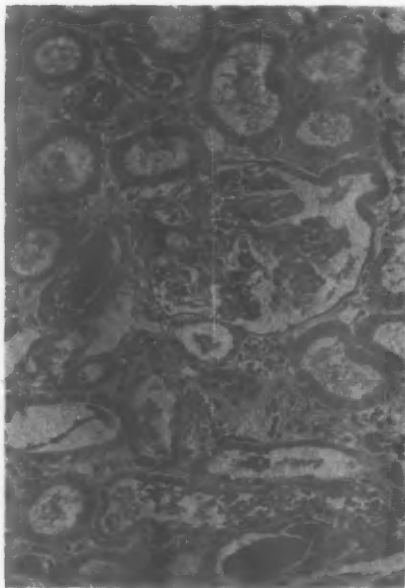


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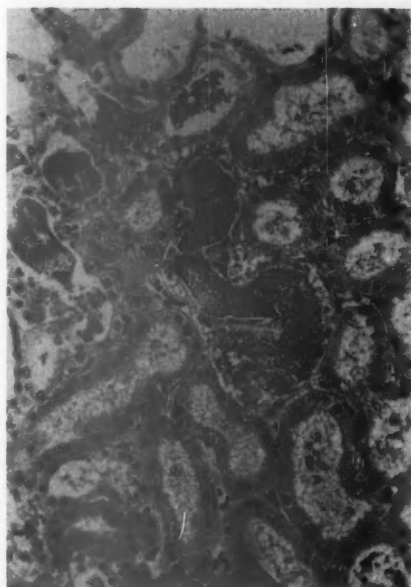


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- FIG. 3. Case 2. Section of cortex, showing well preserved glomeruli with erythrocytes in various loops. Precipitation of albumin in the capsular space and in the proximal convoluted tubules. Hemoglobin casts of varying character are found in the distal convoluted tubules. Hematoxylin and eosin stain.  $\times 160$ .
- FIG. 4. Case 2. A large cast is shown within a distal convoluted tubule, with vacuolation of the tubular cells and nuclear pyknosis. Proximal tubules with albumin in the lumina. Masson-Mallory's stains.  $\times 460$ .
- FIG. 5. Case 2. Cortex with casts of varying aspects, some of them compact and hyalinized. Hematoxylin and eosin stain.  $\times 200$ .
- FIG. 6. Case 2. The large cast shown in Figure 5 under higher magnification. Intense vacuolation and other degenerative changes in the respective tubule (intermediate), with disappearance of some of the cells (pyknosis and karyorrhexis). Surrounding it, proximal tubules show only cloudy swelling. A part of the brush border may still be seen in one of them. Hematoxylin and eosin stain.  $\times 380$ .
- FIG. 7. Case 2. Boundary or intermediate zone showing various casts in the ascending limbs of Henle (intermediate segment). Intense hyperemia of the capillaries in this zone. Hematoxylin and eosin stain.  $\times 200$ .
- FIG. 8. Case 1. Boundary or intermediate zone. Several intermediate segments with casts in the lumen. Marked inflammatory infiltration in the interstitial tissue, especially of neutrophilic granulocytes. Hematoxylin and eosin stain.  $\times 80$ .



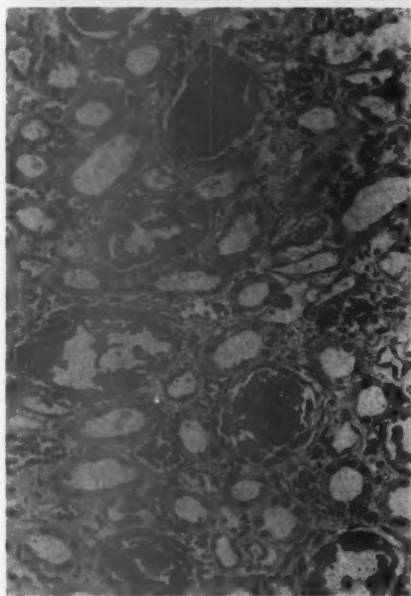
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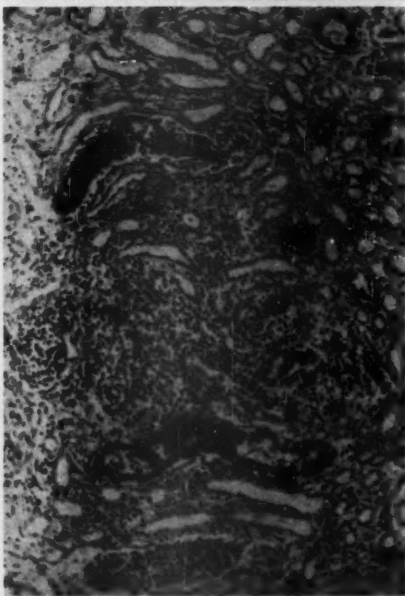


FIG. 9. Case 1. Boundary zone. A cast in ascending limb of the loop with a few neutrophils in the lumen. Intense interstitial inflammatory infiltration of neutrophilic granulocytes with histiocytic proliferation in some areas. Hematoxylin and eosin stain.  $\times 350$ .

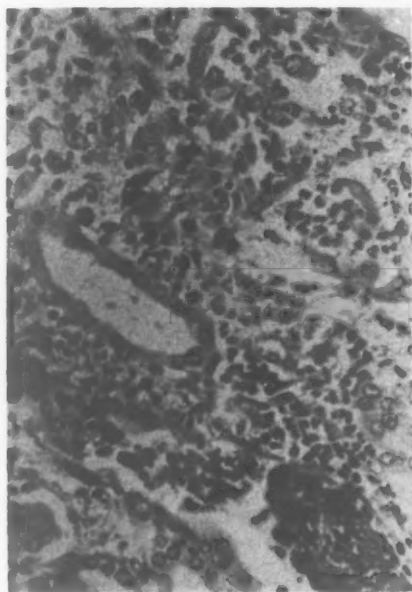
FIG. 10. Case 1. Medulla. Numerous casts in the interior of the collecting tubules with positive dark brown coloration by benzidine (Lepehne method) and light contrasting nuclear coloration by carmine.  $\times 70$ .

FIG. 11. Case 1. Cortex. Glomerular ischemia. Several sections of the distal convoluted tubules and various ascending parts of the loops showing casts. Hematoxylin and eosin stain.  $\times 110$ .

FIG. 12. Case 1. Renal cortex. An epithelial cell with a voluminous chromosome mass, apparently atypical mitosis (proximal convoluted tubule). Above, there is a hemoglobin cast within an intermediate tubular segment. Hematoxylin and eosin stain.  $\times 670$ .

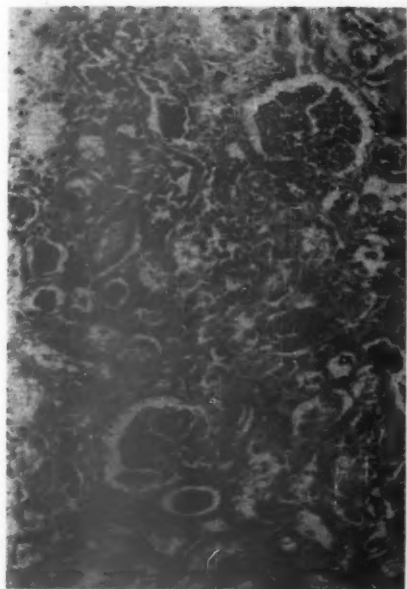
FIG. 13. Case 1. Boundary zone, near the medulla. Various tubules with lumina filled with neutrophil granulocytes near hyaline casts of hemoglobin. Hematoxylin and eosin stain.  $\times 130$ .

FIG. 14. Case 2. Medulla, entirely free from an inflammatory process, shows casts of various aspects in the tubes of Bellini. Hematoxylin and eosin stain.  $\times 130$ .





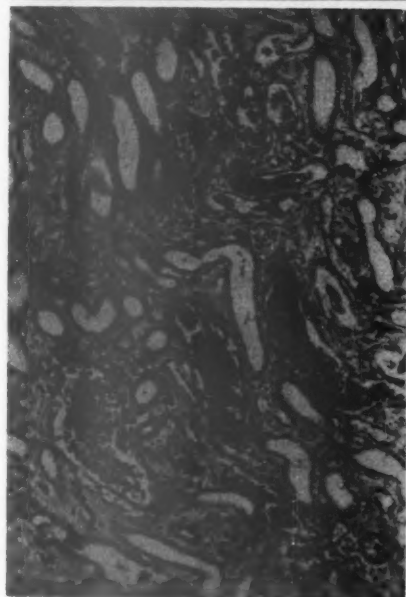
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## THE MORPHOLOGY OF THE MYONEURAL JUNCTION AS INFLUENCED BY NEUROTOXIC DRUGS \*

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Since Doyère<sup>1</sup> in 1840 first described motor end-plates in the striated muscles of insects, this structure has been subjected to continual investigation, marked by three periods during which the pace of investigation was accelerated. In the first period (1840 until about 1903), the names of Kühne, Krause, Ruffini, Ranvier, and Pacini dominated a literature which defined the normal histology of the myoneural junction.<sup>2-4</sup> Dale's<sup>5</sup> work in 1914, on the chemical mediation of nerve impulses at the synapse, provided impetus for a second concentration of studies on the problem, highlighted by the classic interpretations of Wilkinson<sup>6</sup> and the still controversial work of Kulchitsky,<sup>7</sup> Hunter,<sup>8,9</sup> and others who tried to systematize the structural variations of end-plates by identifying them with either efferent, afferent, autonomic, or somatic nerves, as well as with red and white muscle fibers. This phase of study was climaxed by Boeke's comprehensive review of the subject in 1932.<sup>3</sup> Interest then flagged until the 1940's when, because of the German threat during World War II to use anticholinesterase gases in chemical warfare, the end-plate was again subjected to intensive morphologic study and an attempt was made to correlate its structure with physiologic dysfunction.

Better understanding of the morphology *per se* has been achieved within the last 10 years mainly by the prodigious work of Couteaux, and, by the histochemical demonstration of cholinesterase by methods devised by Koelle,<sup>10</sup> Gomori,<sup>11</sup> and Barnett and Seligman.<sup>12</sup> During this same period, however, a voluminous and influential literature was contributed by Carey<sup>13-26</sup> and his collaborators, which contained the remarkable assertions that the motor end-plate functioned like an exocrine gland, that it altered its shape under the influence of various drugs and stresses, and that the gold chloride staining method of Ranvier stained the secretion product of the end-plate, namely, acetylcholine.

Carey's work on the changing dimensions (ameboid motion) of end-plates under various physical and pharmacologic stresses was done by utilizing the gold chloride technique which stains the nerve arborization and which depends upon reduction of the gold salt to its metallic state. With this technique one can see the perinuclear granules of

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Kühne, while the periterminal networks, described as filamentous by Boeke,<sup>3</sup> using the Bielchowski stain, appear as solid oval masses at the end of the arborization. Reducing substances, some of which may be mobile, are responsible for the success of gold chloride as a staining agent.

The brilliant work that Couteaux<sup>26</sup> performed using Janus green B demonstrates that the muscular portion of the myoneural junction (his "subneural apparatus") has a definite form. A structure with identical shape and dimensions can be stained by Koelle's histochemical technique for cholinesterase (Fig. 1). Welsh and Zacks<sup>27</sup> have reported that Janus green B fails to stain the sole plate structure after the muscle has been incubated in diisopropyl-fluorophosphate. This supports the earlier conclusions by Couteaux and Nachmansohn<sup>28</sup> that most of the cholinesterase is situated in the muscular portion of the myoneural junction, specifically at the sole plate, and also suggests that at least part of the total cholinesterase at the junction is arranged in a definite morphologic pattern (Fig. 1).

In the end-plate stained with gold chloride, a non-staining zone (Fig. 2) can be seen around the arborization. After much study and reconstruction of the end-plate, we agree with Denz<sup>29</sup> that this zone is the "synaptic gutter" which partially surrounds the nerve ending filaments, and which is demonstrated as a positive image by Janus green B and Koelle's technique.

Recently, Couteaux and Taxi<sup>30</sup> have demonstrated that Koelle's histochemical technique used at a pH higher than that of 6 as prescribed by Koelle, will demonstrate the neural component. They believed this to be due to migration of the precipitated copper sulfide onto the nerve arborization; this occurred only in tissue that had been previously fixed in formalin. It is singular that formalin fixation confers some sensitivity on nerves for certain metallic impregnations: if silver nitrate, the agent of choice to demonstrate axons after formalin fixation, is substituted for gold chloride in the Ranvier technique in which tissues are fixed in citric acid, no portion of the nerve stains. The neural element is occasionally visible in tissues stained by Koelle's method without altering the pH of the incubating bath, or prefixing in formalin. Figure 3 shows the neural sheath of Henle outlined by copper sulfide. Whatever the cause for the change in staining specificity, however, the demonstration of the neural element by Koelle's stain supports Couteaux's original interpretation that the nerve arborization is superimposed on the cholinesterase "synaptic gutter" which is impressed onto the surface of the muscle fiber.

In the experiments to be described, rat muscles were subjected to various chemical influences; their dimensions, measured under both the gold and Koelle techniques, were compared to control values. It was considered that any evidence of ameboid motion of motor end-plates, as suggested by Carey, should be represented by dimensional changes of the synaptic gutter, as demonstrated by Koelle's technique.

### *Morphologic Methods*

All motor end-plates were demonstrated by the Ranvier gold staining technique as described by Carey,<sup>15</sup> with certain modifications.

1. 5 per cent citric acid, 15 minutes
2. 1 per cent gold chloride, 25 minutes
3. Formic acid, 16 to 24 hours

Instead of small strips of muscle tissue, however, half of the costal cage was immersed in the citric acid solution at room temperature. The attached ribs help to splint the tissue, and to prevent the extreme contraction that occurs in muscle after death. In cases in which the musculus gracilis was sampled, the excised tissue was extended over lucite bridges to its resting length.

Excised parallel specimens were preserved over dry ice until submitted to the cholinesterase technique, when, prior to incubation, samples of muscle were thawed, then teased in saline solution. The solution used for incubation was made up according to the Koelle formula as given by Gomori.<sup>11</sup> Tissue from animals poisoned by anticholinesterases was incubated at 37° C. for 15 and 20 minutes; tissue from control animals and from those poisoned by drugs which have little or no anticholinesterase action was incubated for 10 minutes. Better definition of the image of the synaptic gutter at the sole plate is the ultimate result of decreasing the incubation time; when the enzyme activity is too vigorous, the image is often blurred by diffusion of the reaction end products.

Two series of experiments were performed on albino rats. The first was designed to test the effect on motor end-plates of various drugs injected intramuscularly, and the second to test the effect on motor end-plates of drugs given intravenously.

The drugs used were strychnine, hydrazine, diisopropyl fluorophosphate, tetraethylpyrophosphate, d-tubocurarine (intocostin), and an anticholinesterase compound called ACL.

In the first experiment only specimens of m. intercostalis were examined, and in the second, samples of m. intercostalis and m. gracilis: the latter muscle was added because it was thought that the passive

movements of the chest cage during artificial respiration might influence the structure of the end-plates of the intercostal muscles. The experimental design, detailed in Table I, represents a total of 12 rats, one for each experimental group.

All doses are given in mg. per kg. or ml. per kg., except for those of ACL which are expressed as multiples of the estimated dose which will destroy 100 per cent of the subject animals ( $LD_{100}$ ).

TABLE I  
*Experimental Design*

	Experiment	Drug	Dose (per kg.)	Artificial respiration
<i>Group A</i> Drugs administered intra- muscularly	1	Hydrazine	400 mg.	No
	2	Intocostin	0.5 cc.	No
	3	Strychnine	2.7 mg.	No
	4	Strychnine and intocostin	2.7 mg.; 0.5 cc.	Yes
	5	ACL	$LD_{100} \times 2.5$	No
	6	ACL	$LD_{100} \times 5.0$	Yes
	7	ACL and intocostin	$LD_{100} \times 5$ ; 0.5 cc.	Yes
<i>Group B</i> Drugs administered intravenously (except into- costin given intra- muscularly)	1	Diisopropyl fluorophosphate	100 mg.	Yes
	2	Tetraethylpyrophosphate	105 mg.	Yes
	3	ACL	$LD_{100} \times 8$	Yes
	4	ACL	$LD_{100} \times 10$	Yes
	5	ACL and intocostin	$LD_{100} \times 10$ ; 0.5 cc.	Yes

It should be noted also that, to minimize error from inter-animal variation, in experiment B4 and B5 the m. gracilis from one side was excised before the experiment, and the end-plates were then compared with those of the contralateral muscle excised after completion of the experiment.

Artificial respiration through a tracheal canula was used generally when anticholinesterase agents were administered because these drugs cause interference with normal respiratory function, and death due to anoxia would otherwise ensue. When respiratory death is so obviated, it is possible to give large amounts of the drugs. This accounts for the fact that in animals A5, 6, and 7, and B3, 4, and 5, many times the lethal dose could be given.

Intramuscular administration was accomplished by one injection or two at the most, and if the animal survived it was sacrificed with ether after 30 minutes. Intravenous injections were carried out by adminis-



tering small amounts of the drug every 5 minutes, through a catheter in the femoral vein, until death occurred.

### *Methods of Evaluation*

For the purpose of comparison of the motor end-plates of treated animals with those of untreated controls, at least twenty-four observations, each including three data, were made from each rat. Thus, the long and short diameters of the end-plates and the respective muscle diameters were measured from preparations separately stained by the gold and Koelle techniques. By this method, at least twenty-four sets of end-plate measurements were made for each muscle sample from a single animal. Measurements of the neural arborization from the gold-impregnated tissue, and subneural apparatus from the tissue stained by the Koelle technique were made usually from muscle samples contained on one to three slides. Moreover, many slides representing hundreds of end-plates which were not measured were examined for changes. End-plates to be measured could not be selected completely at random because those which stained indistinctly and those seen from side view had to be eliminated.

White rats weighing 150 to 225 gm. were used throughout the experiment, but despite this, the variation in size of end-plate from one animal to the other was very great. Part of this variation between animals is due to the fact that a direct correlation exists between end-plate diameters and muscle fiber diameters, so that the wider the muscle fiber the larger the end-plate.

Sixty observations from 5 control animals were sufficient to derive an equation which could be used to correct each end-plate measurement to an average muscle diameter, so that variation due to alteration in muscle diameter is cancelled out.

The general form of the equation is as follows:

$$D = D_o - b (M_o - \bar{M}_c)$$

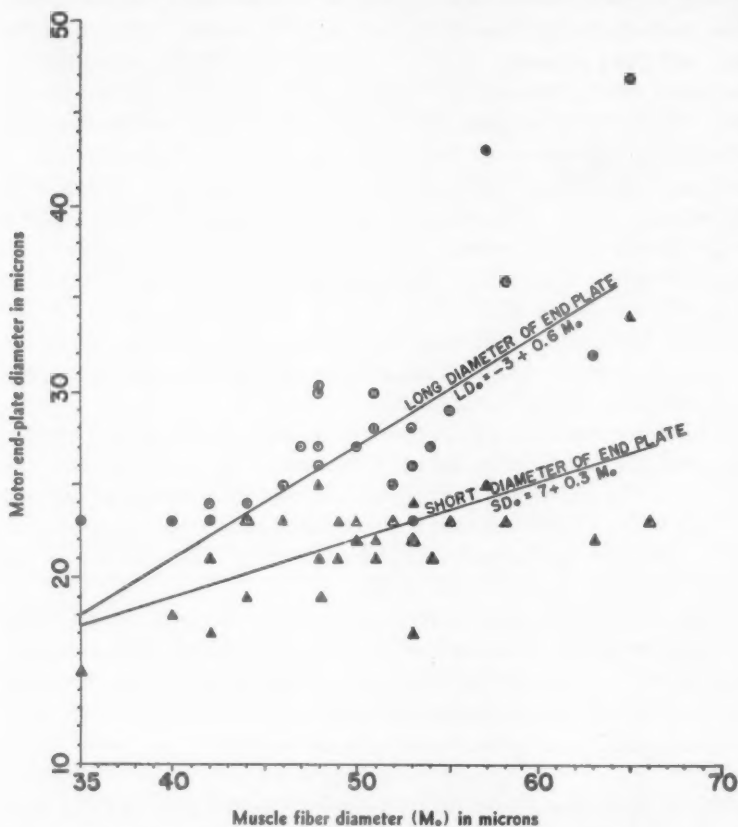
Where  $D$  is corrected end-plate diameter,  $D_o$  is the observed end-plate diameter,  $b$  is the regression coefficient,  $M_o$  the observed muscle diameter, and  $\bar{M}_c$  is the average diameter of control muscles. The curves in Text-figure 1 show the correlation between long and short end-plate diameters and muscle fiber diameter in the intercostal muscle.

### RESULTS

All agents used produced varying degrees of muscle spasm, usually initial clonus which entered a tonic phase before paralysis or death



occurred. Hydrazine exerted the most dramatic effect, stimulating great perpetual activity so that the animal became extremely agitated, performing wild uncontrolled twists and turns, consummated by leaps 2 to 3 feet in the air, and other gyrations requiring strength and agility much beyond the ability of the normal rat. The animals given strychnine



Text-fig. 1. Correlation of motor end-plate diameters with muscle fiber diameters in rat intercostal muscle.

nine developed tonic rigidity of all muscles, including the intercostal group, with death preceded by opisthotonus. Animals treated with anticholinesterase agents generally developed trembling and fibrillary twitches, which were succeeded by clonic and tonic spasticity as the dose increased, often with resultant loss of motor function. The heart rate slowed initially, and subsequent acceleration signalled the onset

of anoxia. Terminal arrhythmias were common. Tenacious bronchiolar secretions often complicated the efforts at artificial respiration, so that the pressure of the respirator had continually to be augmented as the dose was increased. Despite this measure, however, some animals suffocated because of intrapulmonary obstruction. When anoxia was not directly implicated, the cause of death was unknown. Animals given

TABLE II  
*The Effect of Various Drugs on the Dimensions of the Motor End-Plate*

Intercostal muscle*							Gracilis muscle†					
Gold stain				Koelle stain			Gold stain			Koelle stain		
Experimental group	No. of observations	Long diameter	Short diameter	No. of observations	Long diameter	Short diameter	No. of observations‡	Long diameter	Short diameter	No. of observations	Long diameter	Short diameter
Control§	60 (a)	30 ± 7	22 ± 1	72 (b)	25 ± 3	24 ± 2	48 (c)	48 ± 5	29 ± 4	60 (d)	36 ± 4	29 ± 2
A1	12	21	16	45	30	23						
A2	12	25	20	45	28	22						
A3	12	26	22	37	26	24						
A4	12	30	22	45	24	23						
A5	12	30	21	45	25	23						
A6	12	31	18	25	28	24						
A7	12	27	18	46	29	23						
B1	12	27	19				12	45	27	46	27	23
B2	12	33	23				12	55	30	45	51	34
B3	12	33	20	12	30	24	12	63	24	47	37	34
B4	12	25	21				12/12	43/43	26/26			
B5	12	28	20				12/12	48/51	30/30			

\* All end-plate diameters corrected for average muscle diameter of 50  $\mu$ .

† All end-plate diameters corrected for average muscle diameter of 75  $\mu$ .

‡ Treatments B4 and B5 are divided into pretreatment measurements of one gracilis and post-treatment measurements of the contralateral gracilis.

§ Expressed as mean value  $\pm$  the standard deviation. (a) 5 animals; (b) 6 animals; (c) 4 animals; (d) 5 animals.

intocostarin twitched, and often had abortive convulsive movements which persisted for a few seconds before the paralytic phase commenced.

In all experiments the generalized effects of the drugs were allowed to persist for at least 30 minutes, during which time marked muscular activity was observed. Table II shows the results of this study. After all end-plate diameters are corrected for variation in muscle fiber diameter, their distribution appears to be random and indicates no significant differences between experimental groups. In no instance are there consistent changes. When there appears to be a change from control values in end-plate dimension, such as in the gold-stained

arborization of animals treated with hydrazine (Table II), the Koelle preparation of adjacent end-plates shows change in the opposite direction. Significant in this respect is the lack of difference between pre-treatment and post-treatment samples of the *m. gracilis* taken from opposite sides of the same animal in experiments B<sub>4</sub> and B<sub>5</sub>. Certainly no qualitative differences between treated and control animals could be determined by microscopic examination of multiple muscle preparations.

Injections of intocostarin, sufficient to paralyze the animals completely, had no effect on end-plates when this drug was used alone or in conjunction with other drugs. It should be noted, however, that in this experiment the average diameter of the intercostal muscle fibers ( $50\ \mu$ ) differed markedly from that of the *m. gracilis* ( $75\ \mu$ ), and that often the structure as well as the size of the end-plates in the respective muscles varied when stained by the gold technique (Figs. 4 and 6). This difference in structure did not manifest itself in specimens stained by the Koelle technique, although the difference in size was apparent.

Furthermore, no distinct change in aurophilia was produced which could possibly be related to drug action. The cystic bulges at the termination of the efferent neuron, which were described by Carey as being concerned with the "jet-pump" action of the nerve, were never observed.

#### DISCUSSION

The influence of Carey's work has been greater among workers in such fields as pharmacology and physiology<sup>31,32</sup> rather than in pathology. An illusory attitude about the specificity and precision of histochemical techniques, and a lack of recognition of the capriciousness of metallic impregnation techniques, may have led many to accept Carey's net conclusions without reserve.

The following criticisms of Carey's work might be justified:

Normal variation is not defined but is so large that chance alone could have accounted for his results. Although very large numbers of animals were used, representing many thousands of observed end-plates, no statistical evaluation of the results is presented other than unconvincing changes in percentages. The change in diameter of the end-plates as a function of treatment is emphasized, yet there is no mention of the intramuscular, intermuscular, and inter-animal variation in end-plate size, which can be only partially compensated by correcting for differences in muscle diameter.

The end-plate dimensions, especially the longer one, can be correlated with the diameter of the muscle fiber it innervates. From the curve in Text-figure 1, the end-plate diameter can be predicted roughly from any given muscle fiber diameter. Carey does not take this into consideration, so that if the muscle bundles in treated and

control animals happen by chance to have fibers of different diameters, the end-plate diameters will reflect this change.

Although Carey does mention that various muscle groups are used, it is not stated whether they were examined as a homogeneous entity, or whether the different groups were matched for pre-experimental and post-experimental comparison.

In favor of Carey's hypothesis several facts can be presented:

There is precedent in the literature for some of his conclusions.<sup>33-35</sup> The experiments he performed were always reproducible in his hands and in those of his assistants.

Despite the lack of statistical evaluation of the results, the changes reported in the large number of animals certainly seem to represent a trend.

Denz<sup>29</sup> emphatically controverts Carey's conclusions, and his statement is worthy of quotation in its entirety:

"The acceptance of the view that toxic agents can produce characteristic changes in myoneural junctions has usually been based on one or other of Carey's papers and not on a critical assessment of the available evidence, which, taken as a whole, does not support either the hypothesis that morphological lesions have been produced by a variety of toxic agents, or the ancillary suggestion that the great variability of myoneural junctions in normal animals is the consequence of their varying physiological states."

Denz<sup>29</sup> failed to observe morphologic alterations to anticholinesterase agents in rat muscle when these structures were studied by three different methods—gold, silver, and methylene blue. The present paper consequently adds a fourth to this group, the Koelle technique, with which no motor end-plate alterations can be observed, either with anticholinesterase agents, d-tubocurarine (intocostrin), or central nervous system stimulants, such as strychnine and hydrazine.

Miura<sup>33</sup> described changes in the motor end-plates of lizards, but not of frogs, after these animals were subjected to chronic curare poisoning. Herzen and Odier<sup>34</sup> (1904) recorded a change in the frog paralyzed with curare, but the end-plates they described may be the "*terminaisons en grappe*" which Wilkinson<sup>6</sup> cited as a normal finding, and perhaps an immature form of end-plate.

Woollard<sup>35</sup> was taken sharply to task by Denz<sup>29</sup> for his description of lesions of motor end-plates in beriberi and in inanition; Rogers, Pappenheimer, and Goettsch<sup>36</sup> failed to find alterations in the silver-stained end-plates of guinea-pigs which had developed muscular dystrophy ascribed to vitamin E deficiency. Dublin, Bede, and Brown,<sup>37</sup> using Carey's technique, found lesions in the gold-stained end-plates in specimens of muscle taken for biopsy from patients with poliomyelitis, but at least one end-plate (their Figure 8) is no different than one of the many normal variants that can be seen. Chor,<sup>38</sup> who

obtained similar results with silver stains, believed that results with the gold chloride technique were too variable to be reliable.

The gold stain, as do all stains, represents a histochemical reaction. That we are unable to define the substance or substances which react with the stain does not mitigate this fact.

As seen in Figures 1 and 2, the Koelle technique and the gold technique do not outline the same anatomical structure, and generally that which is unstained by the gold is brought out with Koelle's technique and *vice versa*. Although nuclear staining, so common in the Koelle and even in alkaline phosphatase techniques (both of which depend finally upon deposition of metallic compounds), is thought to be an artifact,<sup>10,11,89</sup> it is interesting to note that even with respect to nuclei, the Koelle and gold techniques stain different structures. Figure 5 shows the perinuclear staining by the gold method. Similar condensations of gold at the myoneural junction, the so-called granules of Kühne, are probably related to the nuclei of the sole just as the aurophilic granules and nuclei are related in Figure 5.

Although we never have been able to correlate changes in the amount of perinuclear staining with changes in physiologic status of muscle, these condensations represent either an increased concentration of some metabolite around the nuclei or, perhaps, precipitations of uncharged colloidal particles by substances of opposite charge.

Slight insight into some of the mechanisms involved in metallic impregnation phenomena has been gained through the recent work of Board,<sup>40</sup> who demonstrated the ability of specific substances in tissue to reduce the silver ion in a photographic plate, and we have demonstrated that gold chloride emulsions will react to unfixed tissues placed in contact with them so that a "picture" of the tissue slice will result. Of further interest in this respect is the fact that silver nitrate, when this compound is substituted for gold chloride in the Ranvier technique, is reduced by the muscle but does not deposit on the nerve or the end-plate. This suggests that the reactions in the nerve, end-plate, and muscle may be due to different reducing substances.

If the reduction of gold is dependent upon reducing systems in the muscle and nerve, it is possible that in conditions of extreme muscular fatigue or long-standing paralysis alterations in the reducing potential of nerve and muscle tissue may occur. Changes, such as those described by Carey, then would depict changes in the chemical composition and reactivity of these tissues rather than anatomical alterations.

Figure 7 shows a muscle preparation which was overstained by allowing it to remain in gold chloride for 80 minutes. The gold is

clumped in coarse masses and fails to demonstrate fine structure. A similar change is found after pretreatment of the muscle with acetone for 30 minutes either at 4° C. or at room temperature, before submitting it to the gold technique (Figs. 8 and 9). Some of the changes, described by Carey, resemble the coarse gold deposition that can be achieved either by overstaining or by pretreatment of the tissue with acetone. Conceivably the technique in Carey's hands was sufficiently critical so that the least change in muscle chemistry could be correlated with the tinctorial effects achieved with gold. We have not been able to achieve such delicate balance of the technique in this laboratory.

Changes in muscle response to Koelle's technique as a result of preimmersion of the muscle in acetone at -15° C. were recently reported in the Russian literature.<sup>41</sup> A picture similar to our Figure 9 is shown. Since Koelle's demonstration of cholinesterase is dependent upon a metallic precipitation, it is possible that the pretreatment of muscle by various chemicals could cause changes, the final result of which would be to alter the size of the copper sulfide particles so that unusual sites of deposition would occur. Thus, hypothetically, staining variation might be contingent upon biochemical change secondary to physiologic alteration in the tissue.

Although the theoretic basis of Carey's conclusions, namely, that lesions of the myoneural junction as demonstrated by the gold stain do occur and reflect biochemical change, may well be valid, application of this technique as a biochemical or precise morphologic indicator cannot be justified until there has been more penetrating investigation of the dynamics and physics of the metallic colloids and their interaction with body tissues.

#### SUMMARY

Morphologic alterations were not observed in the motor end-plates of animals subjected to various neurotoxic drugs. Variation in size and structure of motor end-plates as demonstrated by the gold chloride technique is so large in untreated animals that its use as a diagnostic aid is implausible.

Statistical analyses were performed by I. A. De Armon.

#### REFERENCES

1. Doyère, M. Mémoire sur les Tardigrades. *Ann. d. Sc. nat.*, 1840, 14 (Zool.), 269-361.
2. Huber, G. C., and DeWitt, L. M. A. A contribution on the motor nerve-endings and on the nerve endings in the muscle-spindles. *J. Comp. Neurol.*, 1897-98, 7, 169-230.



3. Boeke, J. Nerve Endings, Motor and Sensory. In: Penfield, W. (ed.) Cytology and Cellular Pathology of the Nervous System. Paul B. Hoeber, Inc., New York, 1932, 1, 243-315.
4. Ramón-Cajal, S. Histology. Bailliere, Tindall & Cox, London, 1933, p. 321.
5. Dale, H. H. The action of certain esters and ethers of choline, and their relation to muscarine. *J. Pharmacol. & Exper. Therap.*, 1914-15, 6, 147-190.
6. Wilkinson, H. J. The innervation of striated muscle. *M. J. Australia*, 1929, 2, 768-793.
7. Kulchitsky, N. Nerve endings in muscles. *J. Anat.*, 1924, 58, 152-159.
8. Hunter, J. I., and Latham, O. A contribution to the discussion of the histological problems involved in the conception of a somatic and sympathetic innervation of voluntary muscle. *M. J. Australia*, 1925, 1, 27-36.
9. Hunter, J. I. The sympathetic innervation of striated muscle. *Brit. M. J.*, 1925, 1, 197-201.
10. Koelle, G. B. The elimination of enzymatic diffusion artifacts in the histochemical localization of cholinesterases and a survey of their cellular distributions. *J. Pharmacol. & Exper. Therap.*, 1951, 103, 153-171.
11. Gomori, G. Microscopic Histochemistry. University of Chicago Press, Chicago, 1952, p. 209.
12. Barnett, R. J., and Seligman, A. M. Histochemical demonstration of esterases by production of indigo. *Science*, 1951, 114, 579-582.
13. Carey, E. J., Massopust, L. C., Zeit, W., and Haushalter, E. Studies on ameboid motion and secretion of motor end-plates. VII. Experimental pathology of the secretory mechanism of motor end-plates in thermal shock. *Am. J. Path.*, 1946, 22, 175-233.
14. Carey, E. J., Haushalter, E., Massopust, L. C., Garofalo, F., Lynch, J., Tabat, D., and Socoloff, E. Studies on ameboid motion and secretion of motor end-plates. X. Effects of slow nervous action of disuse on the structure of nerve endings, neurosomes, and muscle fibers. *Am. J. Path.*, 1948, 24, 135-175.
15. Carey, E. J., Massopust, L. C., Haushalter, E., Sweeney, J., Saribalis, C., and Raggio, J. Studies on ameboid motion and secretion of motor end-plates. VIII. Experimental morphologic pathology of the chemical transmitter of nerve impulses in the course of wallerian degeneration. *Am. J. Path.*, 1946, 22, 1205-1285.
16. Carey, E. J., Massopust, L. C., Zeit, W., Haushalter, E., Hamel, J., and Jeub, R. Studies on ameboid motion and secretion of motor end-plates. VI. Pathologic effects of traumatic shock on motor and sensory nerve endings in skeletal muscle of unanesthetized rats in the Noble-Collip drum. *Am. J. Path.*, 1945, 21, 935-1005.
17. Carey, E. J. Studies on ameboid motion and secretion of motor end-plates. IV. Anatomic effects of poliomyelitis on the neuromuscular mechanism in the monkey. *Am. J. Path.*, 1944, 20, 961-995.
18. Carey, E. J. Studies on ameboid motion and secretion of motor end-plates. III. Experimental histopathology of motor end-plates produced by quinine, curare, prostigmine, acetylcholine, strychnine, tetraethyl lead and heat. *Am. J. Path.*, 1944, 20, 341-393.
19. Carey, E. J. Studies on ameboid motion of motor nerve plates. II. Pathologic effects of CO<sub>2</sub> and electricity on the explosive ameboid motion in motor nerve plates in intercostal muscle. *Am. J. Path.*, 1942, 18, 237-289.
20. Carey, E. J., Downer, E. M., Toomey, F. B., and Haushalter, E. Morphologic effects of DDT on nerve endings, neurosomes, and fiber types in voluntary muscles. *Proc. Soc. Exper. Biol. & Med.*, 1946, 62, 76-83.



21. Carey, E. J., Massopust, L. C., Zeit, W., Haushalter, E., and Schmitz, J. Acute anatomic breakdown of motor end plates in hemorrhagic shock. *Proc. Soc. Exper. Biol. & Med.*, 1944, **56**, 115-118.
22. Carey, E. J., and Massopust, L. Sudden destruction of motor end plates by lactic acid. *Proc. Soc. Exper. Biol. & Med.*, 1944, **55**, 194-197.
23. Carey, E. J. Morphologic effects of poliomyelitis virus upon motor end plates in the monkey. *Proc. Soc. Exper. Biol. & Med.*, 1943, **53**, 3-5.
24. Carey, E. J., Massopust, L. C., Zeit, W., Haushalter, E., and Schmitz, J. Studies on ameboid motion and secretion of motor end-plates. V. Experimental pathologic effects of traumatic shock on motor end-plates in skeletal muscle. *J. Neuropath. & Exper. Neurol.*, 1945, **4**, 134-145.
25. Carey, E. J., Massopust, L. C., Zeit, W., and Haushalter, E. Anatomic changes of motor nerve endings in human muscles in early poliomyelitis. *J. Neuropath. & Exper. Neurol.*, 1944, **3**, 121-130.
26. Couteaux, R. Contribution à l'étude de la synapse myoneurals. *Rev. canad. de biol.*, 1947, **6**, 563-711.
27. Welsh, J. H., and Zacks, S. Concerning the significance of the affinity of motor end-plates for Janus green B. (Abstract.) *Anat. Rec.*, 1949, **105**, 526.
28. Couteaux, R., and Nachmansohn, D. Cholinesterase at the end-plates of voluntary muscle after nerve degeneration. *Nature, London*, 1938, **142**, 481.
29. Denz, F. A. Myoneural junctions and toxic agents. *J. Path. & Bact.*, 1951, **63**, 235-247.
30. Couteaux, R., and Taxi, J. Recherches histochimiques sur la distribution des activités cholinestérasiqes au niveau de la synapse myoneurale. *Arch. d'anat. micr.*, 1952, **41**, 352-392.
31. Marrazzi, A. Discussion of Welch, J. H. Concerning the mode of action of acetylcholine. *Bull. Johns Hopkins Hosp.*, 1948, **83**, 568-586.
32. McIntyre, A. R. Some physiological effects of curare and their application to clinical medicine. *Physiol. Rev.*, 1947, **27**, 464-477.
33. Miura, M. Untersuchungen über die motorischen Nervenendigungen der quergestreiften Muskelfasern. *Virchows Arch. f. path. Anat.*, 1886, **105**, 129-135.
34. Herzen, A., and Odier, R. Altération des fibres et filaments nerveux par le curare. *Arch. Internat. de physiol.*, 1904, **1**, 364-372.
35. Woollard, H. H. The nature of the structural changes in nerve endings in starvation and in beri-beri. *J. Anat.*, 1926-27, **61**, 283-297.
36. Rogers, W. M., Pappenheimer, A. M., and Goettsch, M. Nerve endings in nutritional muscular dystrophy in guinea pigs. *J. Exper. Med.*, 1931, **54**, 167-169.
37. Dublin, W. B., Bede, B. A., and Brown, B. A. Pathologic findings in nerve and muscle in poliomyelitis. *Am. J. Clin. Path.*, 1944, **14**, 266-272.
38. Chor, H. Nerve degeneration in poliomyelitis. *Arch. Neurol. & Psychiat.*, 1933, **29**, 344-358.
39. Novikoff, A. B. The validity of histochemical phosphatase methods on the intracellular level. *Science*, 1951, **113**, 320-325.
40. Board, F. A. Sulfhydryl detection by histochemography. *J. Cell. & Comp. Physiol.*, 1951, **38**, 377-387.
41. Portugalov, V. V., and Iakovlav, V. A. [Localization of cholinesterase in the striated muscles.] *Doklady Akad. nauk SSSR*, 1951, **78**, 1021-1024.

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[ Illustrations follow ]

## LEGENDS FOR FIGURES

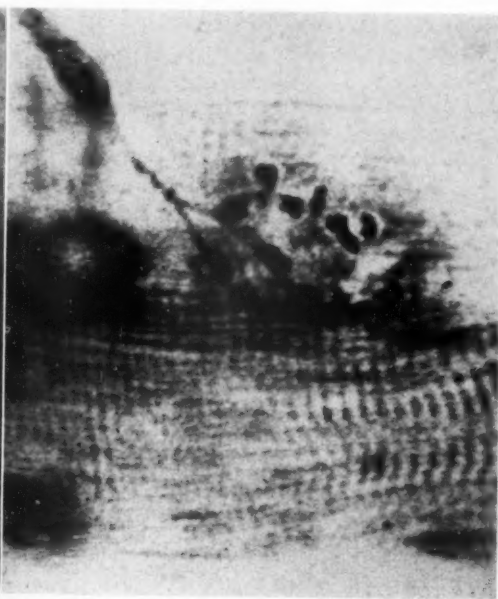
- FIG. 1. Subneural apparatus, or synaptic gutter. Koelle method for demonstration of cholinesterase.  $\times 700$ .
- FIG. 2. Axonal arborization of end-plate demonstrated by gold chloride method.  $\times 700$ .
- FIG. 3. Subneural apparatus and outline of neural sheath of Henle. Koelle method for demonstration of cholinesterase.  $\times 700$ .
- FIG. 4. Large intercostal muscle fiber and neural arborization. Gold chloride method.  $\times 700$ .



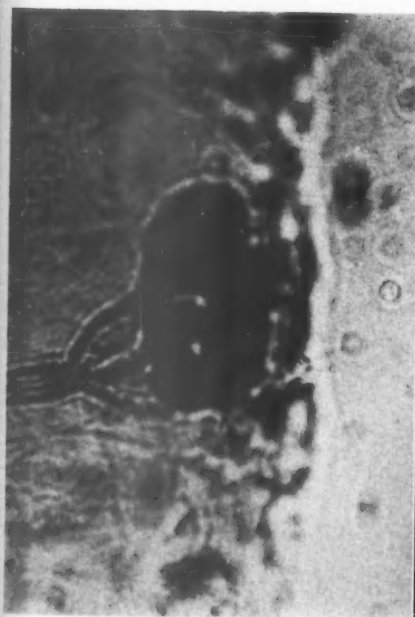




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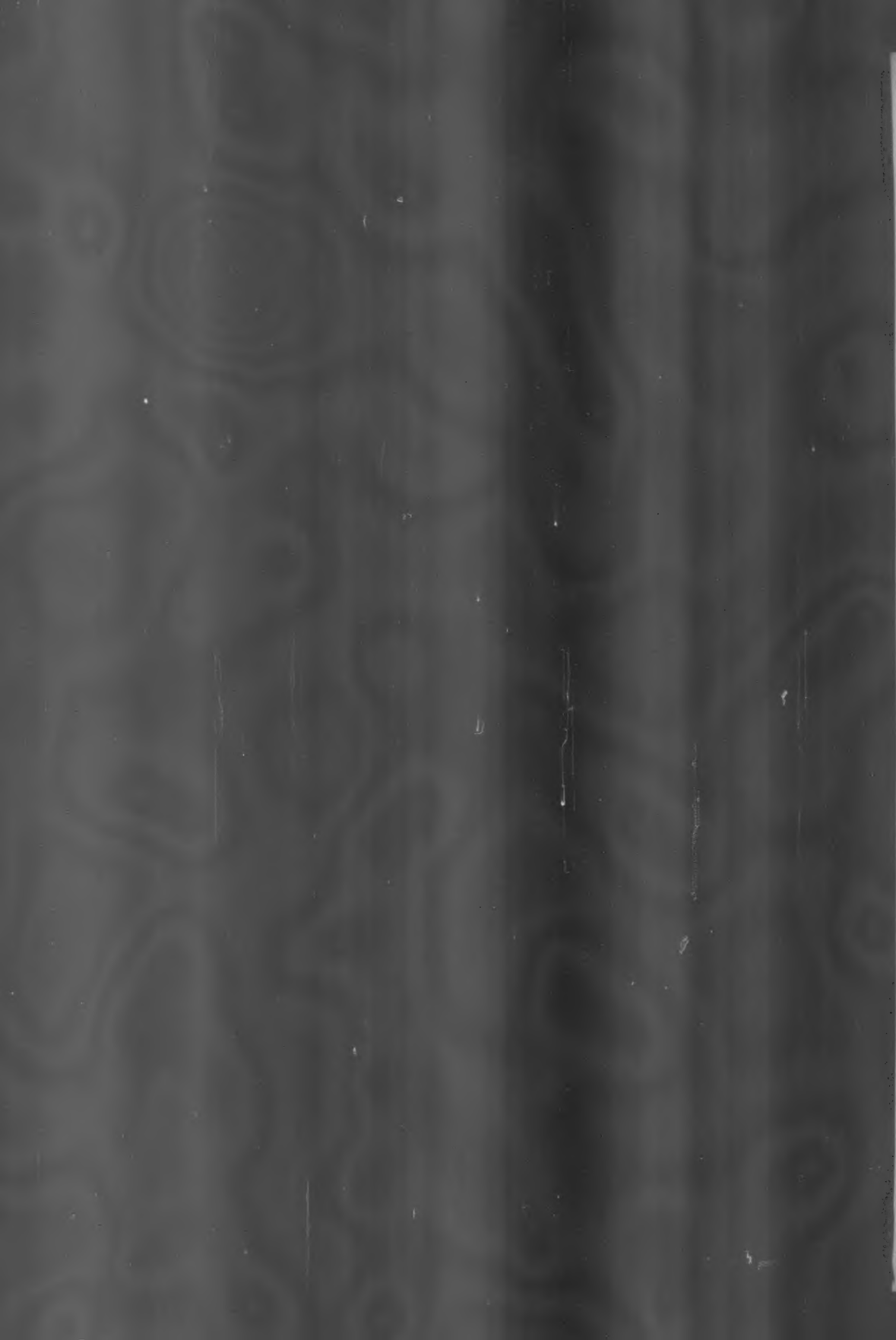
FIG. 5. Perinuclear deposition of gold in muscle fiber. Gold chloride method.  $\times 700$ .

FIG. 6. Axonal arborization of end-plate in posterior gracilis muscle. Gold method.  $\times 700$ .

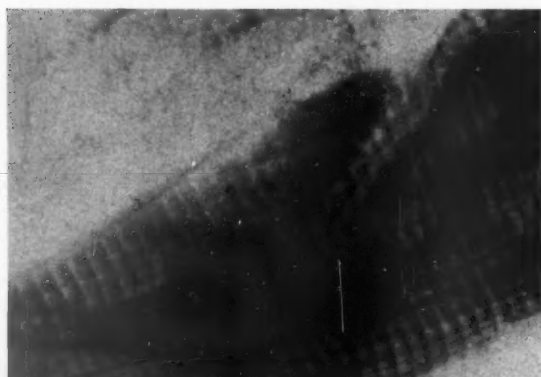
FIG. 7. Nerve and muscle sample overstained by gold chloride method. End-plates are light, or fail to stain.  $\times 240$ .







5



6



7



FIG. 8. Nerve and muscle sample stained by gold chloride technique after initial immersion in acetone at room temperature for 30 minutes. There is intensification of the nerve tree and disappearance of end-plates.  $\times 240$ .

FIG. 9. Clumping of gold in tissue first incubated in acetone at room temperature for 30 minutes.  $\times 240$ .





8



9







## A CHARACTERIZATION OF HYALINE ARTERIOLAR SCLEROSIS BY HISTOCHEMICAL PROCEDURES\*

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Arteriolar sclerosis and benign nephrosclerosis have been considered to be the integral morphologic features of benign hypertension, although the occurrence of similar changes in non-hypertensive subjects, particularly after the age of 50, has been emphasized.<sup>1</sup> In the latter circumstance the vascular sclerosis is less marked and less common than in hypertensive subjects. The position of arteriolar sclerosis in the general problem of hypertension has been stressed by Goldblatt<sup>2</sup> who has considered lack of knowledge of the nature and origin of arteriolar sclerosis as one of the outstanding deficiencies in the understanding of the pathogenesis of essential hypertension.

The correlation between cardiac enlargement and renal contraction depicted by Bright<sup>3</sup> was followed by the description of Johnson<sup>4</sup> of the thickening of small arteries in hypertension and the designation of arterio-capillary fibrosis by Gull and Sutton<sup>5</sup> in 1872. From that time until 1937 descriptions of the vascular lesion of hypertensive cardiovascular disease emphasized mainly thickening of the wall and narrowing of the lumen of arterioles. In 1937 the classical discussion of Moritz and Oldt<sup>6</sup> clarified certain outstanding morphologic features of arteriolar sclerosis. These authors recognized the involvement of various layers of the vessel wall and emphasized particularly three disturbances; namely, endothelial hyalinization, medial hypertrophy and degeneration, and endothelial hyperplasia. Referring to intimal hyalinization, they stated:

"This was the most common form of chronic arteriolar disease observed and consisted of a subendothelial accumulation of homogeneous, acidophilic material which appeared to represent an infiltration or expansion of the ground substance between smooth muscle of the media and the endothelium. . . . Although the principal mass accretion of hyalin was commonly inside of the internal elastic lamella, it appeared that the hyalin actually enveloped the elastic lamella which in many vessels lay approximately in the center of the hyaline mass. In such circumstances, elastic degeneration was invariable and was represented by swelling, disruption and dispersion of fibers with eventual complete disappearance."

The presence of connective tissue elements, as in endothelial hyperplasia<sup>6</sup> and medial fibrosis,<sup>7</sup> appears to be generally agreed upon, but

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the nature of the hyaline substance in the vessel wall remains obscure.

Recently this question has been reviewed and discussed by Duguid and Anderson<sup>8</sup> who, by conventional histologic examination, lent emphasis to the concept that the hyaline substance is derived from the circulating blood. This concept considers that the infiltrate accumulates next to the endothelium and is eventually covered over by the endothelium, while, at the same time, it displaces the normal constituents of the vessel wall, eventuating in a homogeneous replacement of the various layers, associated with thickening of the wall and narrowing of the lumen.

The obscurity of the origin of the hyaline substance is characterized by the treatment of this substance in textbooks. Bell<sup>9</sup> considered that the muscle of the arteriole is replaced by hyalin, but the lumen remains open, and Boyd<sup>10</sup> stated that the appearance suggests an accumulation or deposition of hyaline material which leads to narrowing and, in extreme cases, to complete obliteration of the lumen. It is apparent that an understanding of the source of the hyaline substance in the arteriolar wall represents a necessary requisite to understanding the pathogenesis of this lesion.

Interest in the vascular lesions of hypertensive cardiovascular disease has been revived by observations on the evolution of the arteriolar lesions in the dog following bilateral nephrectomy.<sup>11-14</sup> This experimental preparation has been associated, in our experience, with a high percentage of hypertension and arteriolar lesions, the latter varying because of dependence on several factors, among which has been emphasized the duration of life once the hypertensive state has ensued. Animals succumbing early have demonstrated mainly an explosive necrosis of the smooth muscle of the media of small arteries and arterioles. Animals surviving from 10 to 20 days have demonstrated necrosis of the media of small arteries and arterioles characterized by retainment of the shape of the media, the presence of pyknotic nuclear remnants, and changes which have been interpreted as the early phases of hyalinization. Occasionally in this group of animals, hyalinization of arterioles has been encountered. In those animals surviving beyond 20 days the changes in the wall of the thickened small arteries and arterioles have included total hyalinization of the wall, subendothelial hyalinization with or without medial hyalinization, fibrosis of various layers, and thickening of the internal elastic lamella. By using different stains and observation of the transitional appearances of the vascular lesions it was suggested that the hyaline material is derived from altered smooth

muscle of the media.<sup>12-14</sup> This concept was subsequently strengthened by the use of a battery of histochemical procedures indicating similar findings in the acute lesions of the nephrectomized dog and in the renal arteriolar lesions of "malignant hypertension" of the human.<sup>15</sup> In view of the demonstration of the presence of multiple substances in the walls of altered small arteries and arterioles in the hypertensive cardiovascular disease following bilateral nephrectomy of the dog and malignant hypertension of the human, it was considered worth while to study the hyaline arteriolar sclerosis of benign hypertension of the human by means of similar histochemical procedures. The results of this study are reported in the present communication. We believe they tend to characterize the source of the hyaline substance in the arteriolar sclerosis of human benign hypertension.

### METHODS

The renal tissues studied were obtained fresh at necropsy from four subjects whose pertinent clinical and necropsy findings are given.

### REPORT OF CASES

#### *Case 1*

A white female, 65 years old, died suddenly at home. The right kidney weighed 180 gm.; the left, 110 gm. The surface of each kidney was granular and the cortex thin. The heart weighed 500 gm. and showed coronary arteriosclerosis. Generalized arteriosclerosis was present. Microscopically, the renal lesion consisted of benign arteriolar nephrosclerosis, many small arteries showing hyalinization.

#### *Case 2*

A colored male, 51 years of age, gave a history of shortness of breath and orthopnea of 2 months' duration. His blood pressure when he was first seen in the cardiac clinic was 220/174 mm. of Hg. He was treated in the clinic for hypertensive cardiovascular disease and returned to the hospital because of mental confusion. The blood pressure at that time was 200/146. He expired shortly after admission to the hospital. At necropsy the heart weighed 520 gm. and showed a slight amount of arteriosclerosis. The kidneys weighed 150 gm. each. Their surfaces were finely granular and the capsules stripped with difficulty. The cortex was thinner than normal. Microscopically, the kidneys showed a pronounced degree of benign arteriolar necrosis, superimposed upon which was a moderate degree of acute arteriolar necrosis.

#### *Case 3*

A white female, 70 years old, had been known to have diabetes for 20 years. She had been observed for most of that period in the diabetic clinic where a diagnosis of the Kimmelstiel-Wilson syndrome was made. The blood pressure had been 150/100 mm. of Hg for many years. During the terminal admission she had diabetic acidosis with coma and cardiac failure. Deterioration was rapid. At necropsy the heart weighed 480 gm.; the right kidney, 160 gm.; the left kidney, 140 gm. Both kidneys

displayed coarsely granular surfaces with marked atrophy of the cortex. There was severe generalized arteriosclerosis. Microscopically, the renal lesions consisted of severe benign arteriolar nephrosclerosis with nodular or diabetic intercapillary glomerulosclerosis.

#### *Case 4*

A colored male, 53 years of age, entered the hospital for the fourth time with his chief complaints relating to cardiac failure. He was known to have had hypertension for 3 years, the blood pressure ranging between 190/140 and 160/120 mm. of Hg. The previous admissions had been for congestive heart failure. Demise was due to pulmonary embolism. At necropsy the heart weighed 700 gm. and showed marked subendocardial fibrosis. Generalized arteriosclerosis was prominent. Each kidney weighed 180 gm. The capsules stripped with difficulty, revealing a finely granular surface. Moderate atrophy of the cortex was present. Microscopically, the kidneys showed benign arteriolar nephrosclerosis.

The blocks of tissue were frozen in liquid nitrogen and then kept in the deep freeze until use. Before the sections were cut by the standard frozen section technique the tissue was fixed in the appropriate fixative for the histochemical procedure to be employed. The histochemical procedures employed are described in the standard texts<sup>16,17</sup>; minor variations in the techniques are indicated. Two sets of tissue sections were studied with each histochemical technique. One set was studied by means of the conventional frozen section technique utilizing unfixed tissues. The other set was prepared by a freezing and drying apparatus.\* The lipid stains were performed on material embedded in carbowax after the freeze-drying preparation, while all other procedures were performed on paraffin-embedded tissues. In all cases entirely similar results were obtained. The freezing and drying technique afforded the advantage of preventing diffusion and of allowing the study of serial sections of the same block by a variety of procedures, thus giving continuity to the lesion in question.

The lipid components of the lesion were studied by the following procedures: oil red O, Nile blue sulfate, Schultz, Sudan black B, osmic acid, and Baker's acid hematin reaction with pyridine extraction as a control. Free aldehyde groups were demonstrated by the Schiff procedure. Sulfuric acid esters of polysaccharides were stained by the Congo red amyloid stain. Mucin was stained with Best's mucicarmine stain. Glycogen was stained by Best's glycogen stain. Alkaline and acid phosphatase were demonstrated by the method of Gomori. Free carbonyl groups were demonstrated by the method of Ashbel-Seligman.<sup>18</sup> Potassium was demonstrated by MacCallum's method as

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modified by Gomori.<sup>18</sup> Protein bound sulfhydryl groups were demonstrated by the method of Barnett and Seligman.<sup>19</sup>

### RESULTS

The hyaline material of arteriolar sclerosis is illustrated in Figure 1 after use of Mallory's triple stain. After staining with Verhoeff's stain, Figure 2 shows that the elastic tissue may become fragmented and dispersed throughout the hyaline structure. All of the other figures illustrate the multiplicity of ingredients identified by the histochemical procedures and which will be discussed.

Triglycerides, fatty acids, and phosphatides were demonstrated by positive oil red O stain (Fig. 3), Nile blue sulfate (Fig. 4), and Sudan black B stain (Fig. 5). Acetone extraction failed to modify the Sudan black B stain. The fact that the predominant color in the Nile blue sulfate stain was blue seemed to indicate that the majority of the lipids were present as acidic lipids. Cholesterol and cholesterol esters were present as part of the lipid component as evidenced by the positive Schultz reaction. Although this reaction is not entirely specific for cholesterol, it appears that cholesterol and its esters account for the major portion of the color produced by the reaction. The other lipid stains, as revealed by the osmic acid and Baker's acid hematin reaction, were negative. The Schiff reaction (Fig. 6) was positive, indicating the presence of free aldehyde groups. This reaction was more intense when either frozen or frozen dried tissue was employed, suggesting that part of the positive material consisted of aldehyde groups associated with polysaccharides, mucopolysaccharides, mucoproteins and glycoproteins, glycolipids, and unsaturated lipids; possibly a small fraction resulted from the reaction of aldehyde groups associated with elastic tissue. Alcohol or acetone extraction failed to modify the reaction. The positive Congo red amyloid stain (Fig. 7) indicated the presence of sulfuric acid esters of a polysaccharide, thus offering confirmatory evidence of the presence in the lesions of polysaccharide complexes. Free carbonyl groups were present as demonstrated by the positive reaction employing the Ashbel-Seligman technique<sup>18</sup> (Fig. 8). Potassium was present in the lesions as demonstrated by Gomori's modification of MacCallum's method (Fig. 9). Protein-bound sulfhydryl groups were present in the hyalin as demonstrated by the method of Barnett and Seligman<sup>19</sup> (Fig. 10). The lesions did not contain acid or alkaline phosphatase by Gomori's method, mucin by Best's mucicarmine stain, glycogen by Best's glycogen stain, non-spe-

cific esterase by the Nachlas and Seligman method,<sup>20</sup> desoxyribose nucleic acid by the Feulgen procedure, nor vitamin C.

These histochemical procedures are not considered to be quantitative. It would appear, however, that the hyaline lesions gave more intense reactions with the following procedures than normal arterioles or smooth muscle: oil red O, Nile blue sulfate, Sudan black B, Schultz', Schiff's, Congo red, and potassium. The hyaline substance gave the same reaction for free carbonyl groups and protein-bound sulfhydryl groups as normal arterioles and normal smooth muscle. All of these substances are present in the media of normal arterioles and normal smooth muscle; their apparent increment within the arteriolar lesion in some instances need not necessarily indicate a quantitative increment but may be accounted for as well by an alteration in their physicochemical state as a consequence of the process of hyalinization.

In a previous communication<sup>15</sup> the same battery of histochemical tests was applied to the acutely necrotic arteriole of human malignant hypertension and the necrotic arteriole of the bilaterally nephrectomized dog. In these the histochemical results were identical and with two exceptions the results reported here for the hyaline lesions are identical to those reported for the necrotic arterioles in the previous paper. The most outstanding difference consisted of the presence of acid phosphatase in the acute lesion and its absence in the hyaline lesion.

The second difference resulted from the presence of material giving a positive stain with Sudan black B in the hyaline lesion and its previously reported absence in the acute lesion. The latter difference was discovered to be due to the technique of staining. In the negative case (acute lesion) the procedure for staining was 15 minutes in duration while in the positive instance the stain was developed over a 24-hour period as suggested by Pearse.<sup>21</sup> When the material was restudied using the 24-hour technique, all of the lesions proved to have Sudan black B positive material. Thus the acute human and canine lesions and the hyaline arteriolar sclerotic lesions of man differed histochemically only in the presence of acid phosphatase in the former and its absence in the latter.

#### DISCUSSION

From the results obtained by conventional routine stains and connective tissue stains, and the appearance of transitional changes it has been considered that the hyalin of the small arteries and arterioles in bilaterally nephrectomized dogs is derived in the main from altered necrotic smooth muscle of the media of these vessels.<sup>11-14</sup> It also has



been emphasized that the necrotic lesions of the small arteries and arterioles of nephrectomized dogs are similar to the necrotic lesions of arterioles in malignant hypertension of the human. The resemblance between the lesions of the dog and of man was further strengthened by the demonstration that a battery of histochemical procedures gave the same positive and negative results in these two lesions.<sup>15</sup> Moreover, the substances identified by the histochemical approach could be readily derived from altered smooth muscle. These observations tended to strengthen the view that altered smooth muscle is the source of the hyaline substance in the walls of arterioles.

The present observations on the typical lesion of benign nephrosclerosis, namely, benign arteriolosclerosis or diffuse arteriolar sclerosis, suggest that the hyaline substance is composed of a multiplicity of ingredients, some of which have been identified by this study. These ingredients, with one exception, are those which were found in the acute necrotic lesion of the dog following nephrectomy and in the human in malignant hypertension. The one exception is the enzyme acid phosphatase, perhaps the ingredient most eligible to disappear with time. Thus a histochemical similarity is demonstrated between necrotic smooth muscle of the media of small arteries and arterioles and the smooth acidophilic substance known as hyalin in arteriolar sclerosis. These observations suggest that the hyaline substance is not deposited from a hematogenous source, but represents the ultimate fusion of products derived from necrotic smooth muscle and that, therefore, hyalin in arteriolar sclerosis is autochthonous.

Emphasis has been placed on the cholesterol content of these regions by Baker and Selikoff<sup>22</sup> and of the polysaccharide content of similar experimental lesions by Masson and co-workers.<sup>23</sup> The lipid components of this lesion have been known for some time and have been discussed by Fishberg.<sup>24</sup> The present observations confirm and extend these isolated findings by pointing out that the hyaline structure contains a multiplicity of ingredients. It may be adduced that additional components may yet be identified.

The autochthonous origin of the hyalin of arteriolar sclerosis is in keeping with an outstanding feature of this lesion which has been described or depicted photographically or diagrammatically by many workers, namely, the inverse relationship between hyaline deposition and smooth muscle content of the arterial or arteriolar wall. It is common to encounter attenuation or disappearance of the smooth muscle fibers as hyalin accumulates. The demonstrations of Duguid and Anderson,<sup>8</sup> which were considered to support the hematogenous



origin of the hyaline substance, reveal this reciprocal relationship. Moreover, on occasions one can observe hyaline swelling of individual smooth muscle fibers of the media adjacent to a focus of hyaline deposition, a change which may be considered as an additional transitional link indicating the muscular origin of the hyaline substance.

It has been emphasized by others, namely, Moritz and Oldt,<sup>6</sup> that the principal location of the hyaline accumulation in arteriosclerosis is subendothelial as demonstrated by elastic tissue stains. Although there was subintimal hyaline accumulation in many of the arterioles, this hyalin was contiguous with that of the media and in these areas of continuity the elastic fibers of the elastic lamella fragmented and frequently disappeared completely. This observation offers an explanation for the accumulation of hyalin in a subendothelial location, for it appears more plausible to consider the flow of hyalin from the media into the subendothelial zone through the fragmented and broken elastic tissue than to assume a hematogenous source. This explanation is in keeping with the similar histochemical characteristics of the hyalin in both locations.

#### SUMMARY AND CONCLUSIONS

A histochemical characterization of the hyalin of arteriolar sclerosis of benign hypertension has demonstrated the presence of lipids, carbohydrates, free carbonyl groups, protein-bound sulphydryl groups, and free potassium.

Since similar compounds have been identified in normal smooth muscle, it is likely that the compounds identified in the hyaline substance take their origin from the smooth muscle of the media.

An apparent increase of concentration of some of the ingredients, *i.e.*, lipids, carbohydrates, and potassium, in hyalinized arterioles as compared to normal arterioles is considered to result, in all probability, from unmasking of these substances due to an alteration in their physicochemical state during the process of hyalinization.

The suggestion is made that the subendothelial location of hyalin in some instances results from the flow of hyalin of medial origin through the fragmented internal elastic membrane to a sub-endothelial location.

The hyaline lesions of benign arteriolar sclerosis differ histochemically from the previously reported acute arteriolar lesions of malignant hypertension only in the absence of acid phosphatase.

Hyalin is considered to result from an alteration of the smooth muscle of the media of the arteriole.

We wish to thank Mrs. Ovia Walker for the photographs which appear in this article and for her technical assistance.

## REFERENCES

1. Bell, E. T. The Pathological Anatomy in Primary Hypertension. In: Hypertension, a Symposium. E. T. Bell (ed.) University of Minnesota Press, Minneapolis, 1951, pp. 183-198.
2. Goldblatt, H. Anatomical Considerations of Hypertension. In: Bell, E. T. (ed.) Hypertension, a Symposium, University of Minnesota Press, Minneapolis, 1951, pp. 5-21.
3. Bright, R. Cases and observations, illustrative of renal disease accompanied with the secretion of albuminous urine. *Guy's Hosp. Rep.*, 1836, 1, 338-379.
4. Johnson, G. I. On certain points in the anatomy and pathology of Bright's disease of the kidney. II. On the influence of the minute blood-vessels upon the circulation. *Med.-Chir. Tr., London*, 1868, 51, 57-76.
5. Gull, W. W., and Sutton, H. G. On the pathology of the morbid state commonly called chronic Bright's disease with contracted kidney ("arterio-capillary fibrosis"). *Med.-Chir. Tr., London*, 1872, 55, 273-326.
6. Moritz, A. R., and Oldt, M. R. Arteriolar sclerosis in hypertensive and non-hypertensive individuals. *Am. J. Path.*, 1937, 13, 679-728.
7. Andrus, F. C. The relation of age and hypertension to the structure of the small arteries and arterioles in skeletal muscle. *Am. J. Path.*, 1936, 12, 635-652.
8. Duguid, J. B., and Anderson, G. S. The pathogenesis of hyaline arteriolosclerosis. *J. Path. & Bact.*, 1952, 64, 519-522.
9. Bell, E. T. A Text-book of Pathology. Lea & Febiger, Philadelphia, 1952, ed. 7, p. 773.
10. Boyd, W. A Text-book of Pathology. Lea & Febiger, Philadelphia, 1953, ed. 6, p. 365.
11. Muirhead, E. E., Vanatta, J., and Grollman, A. Hypertensive cardiovascular disease. An experimental study of tissue changes in bilaterally nephrectomized dogs. *Arch. Path.*, 1949, 48, 234-254.
12. Muirhead, E. E., Turner, L. B., and Grollman, A. Hypertensive cardiovascular disease. Vascular lesions of dogs maintained for extended periods following bilateral nephrectomy or ureteral ligation. *A. M. A. Arch. Path.*, 1951, 51, 575-592.
13. Muirhead, E. E., Turner, L. B., and Grollman, A. Hypertensive cardiovascular disease. Nature and pathogenesis of the arteriolar sclerosis induced by bilateral nephrectomy as revealed by a study of its tinctorial characteristics. *A. M. A. Arch. Path.*, 1951, 52, 266-279.
14. Muirhead, E. E., Stirman, J. A., Jones, F., Lesch, W., Burns, M., and Fogelman, M. J. Cardiovascular lesions following bilateral nephrectomy of dog. Role of hypertension and other factors on pathogenesis. *A. M. A. Arch. Int. Med.*, 1953, 91, 250-277.
15. Montgomery, P. O'B., and Muirhead, E. E. Similarities between the lesions in human malignant hypertension and in the hypertensive state of the nephrectomized dog. *Am. J. Path.*, 1953, 29, 1147-1155.
16. Gomori, G. Microscopic Histochemistry; Principles and Practice. University of Chicago Press, 1952, 273 pp.
17. Glick, D. Techniques of Histo- and Cytochemistry. Interscience Publishers, Inc., 1949, 531 pp.
18. Ashbel, R., and Seligman, A. M. A new reagent for the histochemical demonstration of active carbonyl groups. A new method for staining ketonic steroids. *Endocrinology*, 1949, 44, 565-583.

19. Barnett, R. J., and Seligman, A. M. Histochemical demonstration of protein-bound sulfhydryl groups. *Science*, 1952, **116**, 323-327.
  20. Nachlas, M. M., and Seligman, A. M. Evidence for the specificity of esterase and lipase by use of three chromogenic substrates. *J. Biol. Chem.*, 1949, **181**, 343-355.
  21. Pearse, A. G. E. *Histochemistry, Theoretical and Applied*. Little, Brown & Co., Boston, 1953, 530 pp.
  22. Baker, R. D., and Selikoff, M. S. The cholesterol of hyaline arteriosclerosis. *Am. J. Path.*, 1952, **28**, 573-581.
  23. Masson, G. M. C., Phahl, G., Corcoran, A. C., and Page, I. H. Accelerated hypertensive vascular disease from saline and renin in nephrectomized dogs. *A. M. A. Arch. Path.*, 1953, **55**, 85-97.
  24. Fishberg, A. M. Anatomic findings in essential hypertension. *Arch. Int. Med.*, 1925, **35**, 650-668.
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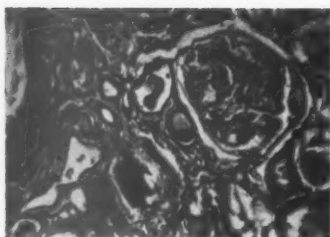
#### LEGENDS FOR FIGURES

- FIG. 1. Case 3. Hyaline arteriole in the kidney. Mallory's triple stain.  $\times 75$ .
- FIG. 2. Case 4. Hyaline arteriole in the kidney. Verhoeff's elastic stain.  $\times 75$ .
- FIG. 3. Case 2. Lipid content of a hyaline arteriole in the kidney. Oil red O stain.  $\times 75$ .
- FIG. 4. Case 2. Lipid content of a hyaline arteriole in the kidney. Nile blue sulfate stain.  $\times 75$ .
- FIG. 5. Case 4. Lipid content of a hyaline arteriole in the kidney. Sudan black B stain.  $\times 75$ .
- FIG. 6. Case 1. Free aldehyde group in a hyaline arteriole in the kidney. Schiff's reaction.  $\times 75$ .
- FIG. 7. Case 3. Polysaccharide complexes in a hyaline arteriole in the kidney. Congo red reaction.  $\times 75$ .
- FIG. 8. Case 4. Free carbonyl groups in a hyaline arteriole in the kidney. Ashbel-Seligman technique.  $\times 75$ .
- FIG. 9. Case 2. Free potassium in a hyaline arteriole in the kidney. Gomori's modification of MacCallum's method.  $\times 75$ .
- FIG. 10. Case 4. Protein bound S-H groups in a hyaline arteriole in the kidney. Barnett and Seligman's method.  $\times 75$ .

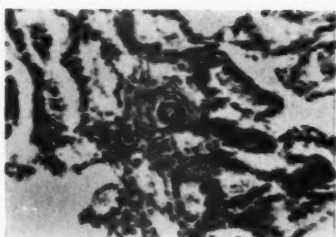




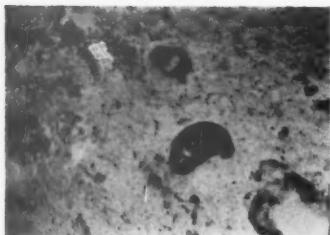
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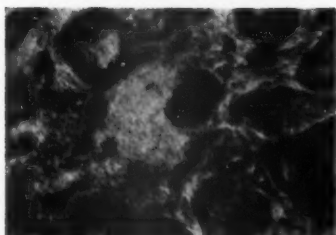
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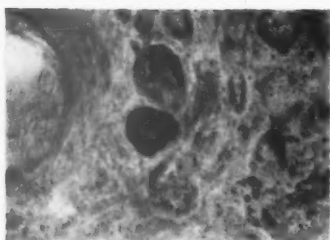
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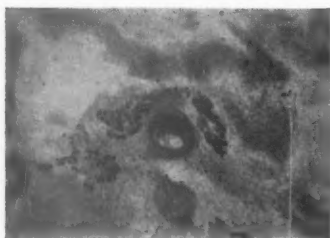
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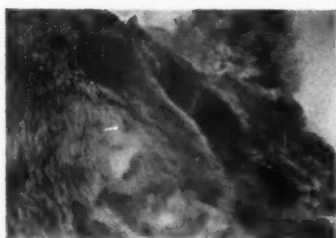
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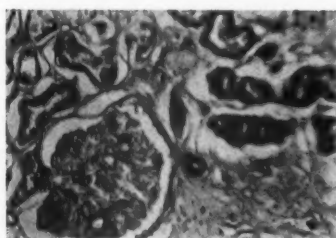
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## A HISTOCHEMICAL STUDY OF THE NEGRI BODIES OF RABIES \*

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Since Negri's<sup>1,2</sup> original observations 50 years ago, there has been little additional information on the nature of the bodies which now bear his name. Although Negri bodies of rabies are of diagnostic importance and are easily identified by their microscopic features, little is known of their chemical structure. Three histochemical studies have been reported,<sup>3-5</sup> but exact information is still lacking as to the nature of these inclusion bodies.

This investigation is a detailed histochemical study of Negri bodies and a study of the physical characteristics of Negri bodies isolated from host cells by homogenization-centrifugation.

### MATERIALS AND METHODS

*Source of Rabies Tissue.* Negri bodies of skunk brain were used throughout most of this study. These Negri bodies are abundant and among the largest found in any species. When absolutely fresh tissues were needed, as for certain enzyme tests, rabies-infected mouse brain was used.

The original rabies virus was obtained from a wild skunk which died from rabies. Brain suspensions from this animal were inoculated into the masseter muscles of two adult, deodorized skunks which were held in confinement until the development of rabies. In the terminal stages of the disease the skunks were killed by decapitation, the brains were removed, and blocks of hippocampus and cerebral cortex were prepared by freezing-drying and with various chemical fixatives.

*Fixation.* Fixatives giving good cytologic detail and a minimum of artifacts were used whenever possible, but in a few cases, such as with certain sensitive enzyme tests, chemical fixation was not permissible and fresh or frozen-dried tissues were used exclusively. Chemical fixation (Table I) was carried out for 24 hours at 4° C. and tissues were prepared for sectioning in the usual manner. Sections were cut at 4, 6, and 10  $\mu$ .

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At various times during the course of the study touch impressions were prepared from fresh rabies-infected skunk and mouse brain. These were fixed immediately in chilled solutions of 80 per cent alcohol, 95 per cent alcohol, acetone or Rossman's fluid. Impressions subjected to enzyme digestion and used in tests for specific enzymes were fixed after treatment.

Skunk brain was also prepared by freezing and drying. Attempts to dehydrate 2 mm. slices of brain tissue were unsuccessful after treatment for 21 hours in the freezing-drying unit, so touch impressions of brain tissue were employed. Impressions were made on coverslips and frozen in isopentane previously cooled to  $-180^{\circ}$  C. by liquid nitrogen. These were transferred to a freezing-drying unit<sup>6</sup> and dehydrated at  $-40^{\circ}$  C. and  $10^{-5}$  mm. of Hg for 8 hours. The impressions were held at  $-30^{\circ}$  C. in a desiccator until needed. Tissues subjected to severe hydrolysis, enzyme digestion, or chemical extraction were covered with a thin coat of celloidin before staining.

*Histochemical Methods.* The various histochemical methods and substances investigated are listed in Table I. In order to avoid false interpretations of histochemical tests, controls were run whenever possible in order to avoid confusing the genuine reaction with other similar reactions of non-specific nature. In a number of cases (for instance, the Prussian blue reaction) the tests were so highly specific that no controls were necessary. Two main methods were used for verification of the specificity of reactions: (1) the omission of essential ingredients in control slides and (2) the use of inactivators, inhibitors, and extractives such as excessive heat, solvents, strong acids, and enzymes, depending on the nature of the substance investigated.

*Isolation of Negri Bodies by Homogenization-Centrifugation.* In addition to the various histochemical tests used, the technique of homogenization and centrifugation in sucrose<sup>7,8</sup> was employed in order to study the physical characteristics of Negri bodies isolated from host cells. The entire process was carried out in a cold room at  $4^{\circ}$  C. One-half gram samples of fresh infected skunk brain were added to 4.5 ml. of 0.88 M sucrose and 0.5 ml. of 0.00054 M calcium chloride and homogenized for 30 seconds in a Waring blender. The material was then centrifuged for 15 minutes at 1000 g. in a horizontal centrifuge and the supernatant was discarded. The sediment was resuspended in ten times its volume of fresh sucrose solution and centrifuged for 15 minutes. This procedure was repeated until the supernatant was clear or until smears of the sediment revealed no granular debris.

## OBSERVATIONS AND DISCUSSION

Histochemical results are indicated in Table I. The ferrocyanide test<sup>9</sup> for proteins showed positive reactions in the Negri body ground substance and inner granules (Figs. 1 and 2). These stained blue and appeared in sharp contrast to the lighter staining cells. The Sakaguchi

TABLE I  
*Histochemical Methods for Particular Substances and Results on Negri Bodies of  
Skunk Brain*

Substance	Methods	Fixation	Results*
Protein	Ferrocyanide <sup>9</sup>	Formalin	+
$\alpha$ -Amino acid	Ninhydrin <sup>13</sup>	Formalin; freeze-dry; fresh	+
Arginine	Sakaguchi <sup>10, 11</sup>	Formalin; Bouin's; freeze-dry	+
Tyrosine	Millon <sup>14, 15</sup>	Formalin; freeze-dry	+
Desoxyribonucleic acid	Feulgen <sup>29</sup>	Formalin; Zenker's; freeze-dry; fresh	+
	Desoxyribonuclease <sup>30</sup>	Freeze-dry; fresh	+
Ribonucleic acid	Ribonuclease <sup>30</sup>	Freeze-dry	-
Glycogen	Periodic acid-Schiff <sup>18</sup>	Formalin; acetone; freeze-dry; fresh	-
	Best carmine <sup>19</sup>	Alcohol-formalin	-
Polysaccharide complexes	Periodic acid-Schiff <sup>18</sup>	Formalin; freeze-dry; fresh	?
Mucopolysaccharides	Metachromasia <sup>30</sup>	Formalin; acetate-formalin; basic lead acetate; freeze-dry	-
	Hyaluronidase <sup>17</sup>	Freeze-dry; fresh	-
Ascorbic acid	Acid silver <sup>21</sup>	Freeze-dry	-
Lipids	Nile-blue sulfate, <sup>23</sup>	Freeze-dry; fresh	-
	Sudan IV, <sup>25</sup>		
	Sudan black B <sup>17</sup>		
Phospholipids	Acid hematein-pyridine extraction <sup>23</sup>	Freeze-dry	-
Cholesterol	Schultz <sup>24</sup>	Freeze-dry	-
Inorganic iron	Prussian blue, Turnbull's blue <sup>24</sup>	Formalin; alcohol; freeze-dry	-
Organic iron	Prussian blue <sup>9, 24</sup>	Formalin; alcohol; freeze-dry	-
	Di-nitrosoresorcino <sup>17</sup>	Formalin	?
Calcium	von Kossa <sup>9</sup>	Formalin	-
	Cretin <sup>26</sup>	Formalin	-
Alkaline phosphatase	Calcium-cobalt <sup>9</sup>	Formalin; acetone; alcohol; freeze-dry	-
Dehydrogenase	Tetrazolium <sup>28</sup>	Freeze-dry; fresh†	-
Cytochrome oxidase	G Nadi <sup>9</sup>	Freeze-dry; fresh†	-

\* A plus sign indicates a positive reaction; a minus sign, a negative reaction.

† Mouse brain tissue.

method<sup>10, 11</sup> for arginine was the most successful of the tests for amino acids in proteins. This test indicated a positive reaction with reddish brown staining of the Negri bodies (Fig. 3). The nuclei appeared a

lighter shade. The Romieu method<sup>12</sup> for tryptophane caused too much tissue destruction to be of any use and the ninhydrin reaction<sup>13</sup> for alpha amino acid groups produced fleeting colors which were difficult to localize. The ninhydrin reaction could be interpreted only in the fresh and frozen-dried preparations and there the Negri bodies and nuclei took the dark purple stain characteristic of a positive test. The Millon test<sup>14,15</sup> produced a reddish brown staining of Negri bodies and lighter staining of nuclei (Fig. 4). This staining was sufficiently pronounced to indicate a positive test for tyrosine in the Negri bodies.

The Negri bodies tested for desoxyribonucleic acid reacted erratically to the Feulgen technique and some showed complete negativity (Fig. 5). This was especially true of the larger and presumably older inclusions in chromatolysed nerve cells. However, Negri bodies also were found with pink, Feulgen-positive, inner granules and colorless ground substance (Figs. 6, 7, and 8).

The fact that all Negri bodies did not react to the Feulgen test does not necessarily indicate that those which did were showing artifactual staining. In the studies of Wolman and Behar,<sup>5</sup> Feulgen positivity was observed only in the early Negri bodies and the test was negative in the older forms. These authors believed that the range of reactivity to the Feulgen method could be explained by the variation in age of the Negri bodies.

Tissues containing Negri bodies were also hydrolyzed in solutions containing desoxyribonuclease\* and subsequently stained by Sellers' method.<sup>16</sup> Frozen-dried and fresh impressions were treated for 10, 30, 45, and 75 minutes at 37° C. and at room temperature with a solution containing 10 mg. of crystalline desoxyribonuclease to 1 ml. of 0.01 per cent magnesium sulfate. Control tissues were treated in substrate without enzyme. Desoxyribonuclease reduced basophilic staining in the inner granules of the Negri body (Fig. 9). This was accompanied by loss of basophilia in nuclear chromatin. Control tissues stained with Sellers' method exhibited Negri bodies with dark blue inner granules and pink ground substance (Fig. 10). In so far as desoxyribonucleic acid is concerned with basophilic staining of the inner granules of the Negri body, desoxyribonuclease is able to reverse it and the results of this test appear to confirm those of the Feulgen reaction with regard to the presence of desoxyribonucleic acid in the inner granules of the Negri body.

The ribonuclease tests for ribonucleic acid in Negri bodies were

\* General Biochemicals, Inc.

consistently negative and these results confirm those of Lépine and Sautter.<sup>4</sup> Frozen-dried tissues were hydrolyzed for 3 hours at 50° C. in 0.1 per cent crystalline ribonuclease\* in veronal buffer<sup>17</sup> (pH, 6.78). Controls were incubated in veronal buffer without the enzyme. Test and control tissues were then stained with toluidine blue O and both showed Negri bodies with colorless ground substance and blue inner granules (Fig. 11).

Negri bodies stained by the periodic acid-Schiff method<sup>18</sup> appeared pink in formalin-fixed sections (Fig. 12) but there was no difference in color in sections treated in a similar manner except for omission of periodic acid oxidation in the procedure (Fig. 13). The color of the Negri bodies in this test was not the purplish pink observed in the epithelium of the salivary gland of the skunk in control sections. When fresh and frozen-dried tissues were treated by Schiff's reagent, the Negri bodies showed faintly perceptible pink staining in the inner granules and colorless ground substances. Attempts to prevent this stainability with various enzymes, extractives, and buffer solutions were unsuccessful because slight variations in staining could not be determined absolutely in the minute granules.

The Best carmine test<sup>19</sup> for glycogen stained inclusion granules pink, but control sections treated with saliva showed similar color. Tests for acid mucopolysaccharides in Negri bodies by the metachromasia method with toluidine blue<sup>20</sup> and by commercial hyaluronidase<sup>17</sup> followed by Sellers' stain were negative. The test for ascorbic acid<sup>21</sup> also was negative.

Neutral fats, phospholipids, and cholesterol could not be demonstrated in Negri bodies by any of the methods employed.<sup>17,22-25</sup>

Inorganic iron<sup>9,24</sup> and calcium<sup>9,26</sup> could not be demonstrated, but the Prussian blue tests<sup>9,24</sup> for organic iron, using nitric acid as an unmasking agent, revealed Negri bodies with positive, blue-staining inner granules (Figs. 14 and 15). Attempts to confirm this test with the di-nitrosoresorcinol reaction<sup>27</sup> for organic iron were unsuccessful because of poor localization. Organic iron has previously been reported in Negri bodies by Covell and Danks.<sup>8</sup>

Frozen-dried preparations tested for alkaline phosphatase by Gomori's calcium-cobalt method<sup>9</sup> showed black precipitate in Negri bodies and nuclei. However, slides treated in a similar manner except for the omission of sodium glycerophosphate in the procedure showed similar black precipitate and there was no quantitative difference between

\* General Biochemicals, Inc.

the two. Wolman and Behar<sup>5</sup> found Negri bodies positive for alkaline phosphatase by Gomori's method, but it is not clear whether they included control slides to check for artifactitious staining. These workers also observed acid phosphatase and cholinesterase in Negri bodies. Histochemical tests for dehydrogenase<sup>28</sup> and cytochrome oxidase<sup>9</sup> in both frozen-dried skunk brain and fresh rabies-infected mouse brain were negative.

Homogenization-centrifugation of the brain of rabid skunks succeeded in isolating many Negri bodies from host cells, and the inclusion bodies along with isolated nuclei formed a gray button at the bottom of the centrifuge tube. These Negri bodies showed no perceptible changes in structure or tinctorial properties. The greatest concentration of isolated Negri bodies was found near the bottom of the sediment, but there was no definite layering-out of inclusions from nuclei. Negri bodies also were found adhering to nuclei even with complete absence of cell cytoplasm. When homogenization or centrifugation was prolonged, Negri bodies and nuclei became fragmented. This always occurred sooner in nuclei than in Negri bodies. A point could be reached in centrifugation where nuclei became too finely dispersed to settle at slow centrifuge speeds and only fragments of Negri bodies could be found at the bottom of the tube. These fragments often adhered in an amorphous mass, but they could still be identified as Negri bodies in stained smears by the presence of inner granules.

In a loopful of inclusion sediment mounted in saline solution, isolated Negri bodies could be identified by definite refractile limiting membranes, coarse and refractile inner granules, and semi-opaque ground substance. They differed from isolated nuclei which showed less conspicuous limiting membranes, finer granulation, greater transparency, and the presence of nucleoli. The isolated Negri bodies tended to settle more rapidly than nuclei in a drop of saline solution and the inclusions showed little tendency to adhere to one another or to the slide surface. When dilute Janus green B stain was pipetted into the drop of saline solution, the Negri body inner granules took up the stain before the ground substance.

Inclusion sediment mounted in 1 per cent solutions of trypsin and pancreatin showed lysis of Negri bodies and nuclei at approximately the same time. Citric acid and distilled water caused swelling of inclusion bodies and nuclei.

#### SUMMARY

The Negri bodies in rabies-infected skunk brain were studied by histochemical and isolation techniques. The Negri bodies showed positive reactions for protein, arginine, tyrosine, and alpha amino acids.



The inner granules of the Negri bodies reacted positively for desoxy-ribonucleic acid and organic iron. Inconclusive results were obtained in the periodic acid-Schiff test for polysaccharide complexes. Negative staining results were obtained for ribonucleic acid, glycogen, hyaluronic acid, mucopolysaccharides, ascorbic acid, neutral fats, phospholipids, cholesterol, inorganic iron, calcium, alkaline phosphatase, dehydrogenase, and cytochrome oxidase. Physical characteristics were studied in Negri bodies isolated from host cells by the technique of homogenization-centrifugation in sucrose.

## REFERENCES

1. Negri, A. Zur Aetiologie der Tollwuth. Die Diagnose der Tollwuth auf Grund der neuen Befunde. *Ztschr. f. Hyg. u. Infektionskr.*, 1903, **44**, 519-540.
2. Negri, A. Beitrag zum Studium der Aetiologie der Tollwuth. *Ztschr. f. Hyg. u. Infektionskr.*, 1903, **43**, 507-527.
3. Covell, W. P., and Danks, W. B. C. Studies on the nature of the Negri body. *Am. J. Path.*, 1932, **8**, 557-571.
4. Lépine, P., and Sautter, V. Étude histochimique des lésions dues aux ultravirus; les acides nucléiques. *Ann. Inst. Pasteur*, 1946, **72**, 174-183.
5. Wolman, M., and Behar, A. A cytochemical study of the nature of Negri bodies. *J. Infect. Dis.*, 1952, **91**, 69-71.
6. Glick, D., and Malmstrom, B. G. Studies in histochemistry. XXIII. Simple and efficient freezing-drying apparatus for the preparation of embedded tissue. *Exper. Cell Research*, 1952, **3**, 125-135.
7. Schneider, R. M., and Petermann, M. L. Nuclei from normal and leukemic mouse spleen. I. The isolation of nuclei in neutral medium. *Cancer Research*, 1950, **10**, 751-754.
8. Petermann, M. L., and Schneider, R. M. Nuclei from normal and leukemic mouse spleen. II. The nucleic acid content of normal and leukemic nuclei. *Cancer Research*, 1951, **11**, 485-489.
9. Gomori, G. *Microscopic Histochemistry*. University of Chicago Press, Chicago, 1952, 273 pp.
10. Baker, J. R. The histochemical recognition of certain guanidine derivatives. *Quart. J. Micr. Sc.*, 1947, **88**, 115-121.
11. Serra, J. A. Histochemical tests for proteins and amino acids; the characterization of basic proteins. *Stain Technol.*, 1946, **21**, 5-18.
12. Romieu, M. Sur la détection histochimique des substances protéiques. *Bull. d'histol. appliq. à la physiol.*, 1925, **2**, 185-191.
13. Berg, W. Zum histologischen Nachweis der Eiweisspeicherung in der Leber. *Arch. f. d. ges. Physiol.*, 1926, **214**, 243-249.
14. Bensley, R. R., and Gersh, I. Studies on cell structure by the freezing-drying method. II. The nature of the mitochondria in the hepatic cell of amblystoma. *Anat. Rec.*, 1933, **57**, 217-237.
15. Pollister, A. W. Quelques méthodes de cytologie chimique quantitative. *Rev. d'hémat.*, 1950, **5**, 527-554.
16. Sellers, T. F. A new method for staining Negri bodies of rabies. *Am. J. Pub. Health*, 1927, **17**, 1080-1081.
17. Herman, E. *Histochemical and Cytological Techniques*. Department of Anatomy, Harvard Medical School, Boston, 1951, 54 pp.
18. McManus, J. F. A. Histological demonstration of mucin after periodic acid. *Nature, London*, 1946, **158**, 202.



19. Bensley, C. M. Comparison of methods for demonstrating glycogen microscopically. *Stain Technol.*, 1939, **14**, 47-52.
20. Wislocki, G. B., Bunting, H., and Dempsey, E. W. Metachromasia in mammalian tissues and its relationship to mucopolysaccharides. *Am. J. Anat.*, 1947, **81**, 1-37.
21. Deane, H. W., and Morse, A. The cytological distribution of ascorbic acid in the adrenal cortex of the rat under normal and experimental conditions. *Anat. Rec.*, 1948, **100**, 127-141.
22. Baker, J. R. The histochemical recognition of lipine. *Quart. J. Micr. Sc.*, 1946, **87**, 441-470.
23. Cain, A. J. The use of Nile blue in the examination of lipoids. *Quart. J. Micr. Sc.*, 1947, **88**, 383-392.
24. Glick, D. *Techniques of Histo- and Cytochemistry*. Interscience Publishers, Inc., New York, 1949, 531 pp.
25. Kay, W. W., and Whitehead, R. The staining of fat with Sudan IV. *J. Path. & Bact.*, 1935, **41**, 303-304.
26. Cretin, A. Sur un nouveau réactif du calcium applicable aux recherches histologiques. *Bull. d'histol. appliq. à la physiol.*, 1924, **1**, 125-132.
27. Humphrey, A. A. Di-nitrosoresorcinol—a new specific stain for iron in tissues. *Arch. Path.*, 1935, **20**, 256-258.
28. Black, M. M., Opler, S. R., and Speer, F. D. Observations on the reduction of triphenyl tetrazolium chloride by normal and malignant human tissue. *Am. J. Path.*, 1950, **26**, 1097-1102.
29. Stowell, R. E. Feulgen reaction for thymonucleic acid. *Stain Technol.*, 1945, **20**, 45-58.
30. Brachet, J., and Shaver, J. R. The effect of nucleases on cytochemical reactions for amino acids and on staining with acid dyes. *Stain Technol.*, 1948, **23**, 177-184.

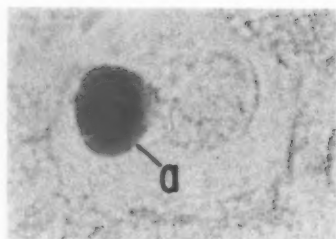
#### LEGENDS FOR FIGURES

- FIGS. 1 and 2. Hartig-Zacharias ferrocyanide test for protein. Negri bodies (a) show positive staining inner granules and ground substance. Nuclei (to right of Negri body in Fig. 1 and below in Fig. 2) stain much lighter. No counterstain.  $\times 2000$ .
- FIG. 3. Sakaguchi reaction for arginine. Negri body (a) shows positive staining. Nucleus (to left of inclusion) is faintly stained. No counterstain. Formalin fixation.  $\times 2000$ .
- FIG. 4. Millon reaction for tyrosine. Negri body (a) stains dark. Nucleus is faintly perceptible. No counterstain. Formalin fixation.  $\times 1800$ .
- FIG. 5. Feulgen reaction for desoxyribonucleic acid. Negri body (a) (the large pale mass occupying entire left half of cell) shows negative staining. Nucleus (to right of inclusion) contains Feulgen-positive chromatin. There is a halo of positive chromatin around the nucleolus. No counterstain. Formalin fixation.  $\times 2500$ .
- FIGS. 6, 7, and 8. Feulgen reaction for desoxyribonucleic acid. Negri bodies (a) contain Feulgen-positive inner granules. Inner granules are within vacuoles in Figures 6 and 8. Nuclei (left of inclusion in figures) show Feulgen-positive chromatin. No counterstain. Formalin fixation.  $\times 1800$ .

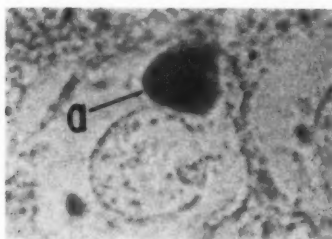




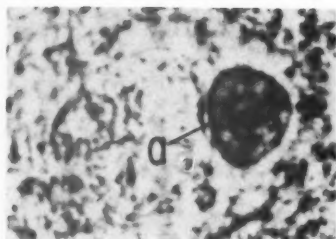
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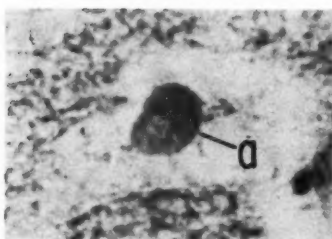
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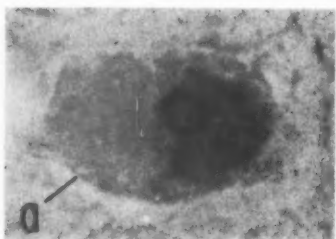
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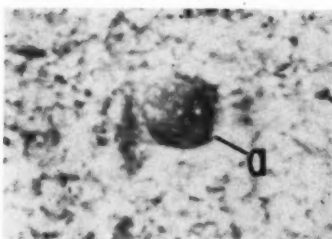
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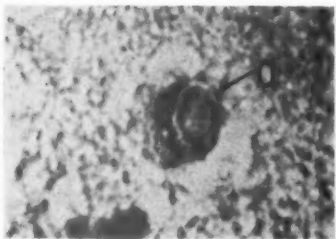
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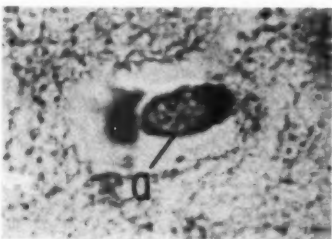
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FIGS. 9 and 10. Desoxyribonuclease test for desoxyribonucleic acid. Negri body (a) in Figure 9 has been digested by desoxyribonuclease and subsequently stained by Sellers' method. There is loss of staining of inner granules. Negri body (a) in Figure 10 was treated simultaneously except for omitting enzyme digestion. It shows dark-staining inner granules and central mass. Nuclei (left of inclusions in both figures) show comparable changes in staining. Freeze-dry.  $\times 2500$ .

FIG. 11. Toluidine blue O. Negri body (a) exhibits specific staining of inner granules and central mass. Freeze-dry.  $\times 2500$ .

FIGS. 12 and 13. Periodic acid-Schiff's test for polysaccharide complexes. Negri bodies (a) in both figures have been treated the same except for omission of periodic acid oxidation in the test procedure in Figure 13. The two Negri bodies show little difference in staining and the reaction is considered non-specific. Hematoxylin counterstain. Formalin fixation.  $\times 2500$ .

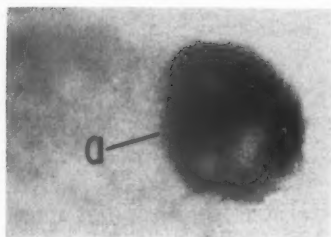
FIGS. 14 and 15. Prussian blue test for organic iron. Iron unmasked with Macallum's reagent. Inner granules of Negri bodies (a) appear as small halos and show specific staining. No counterstain. Formalin fixation.  $\times 1800$ .



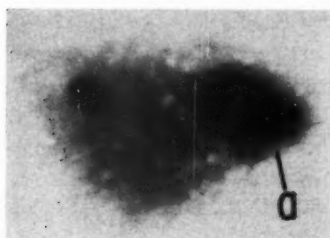




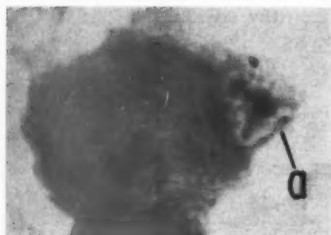
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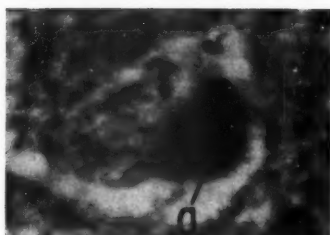
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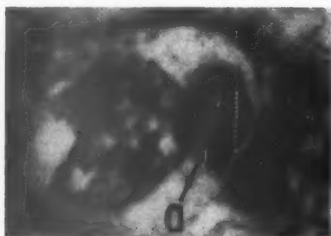
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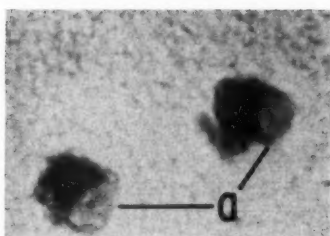
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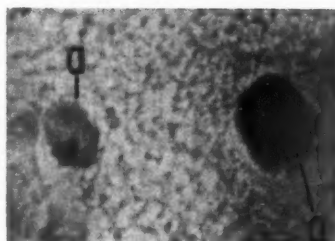
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## ANISOTROPIC CRYSTALS IN THE HUMAN THYROID GLAND \*

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Anisotropic crystals have been reported in the thyroid gland by several observers. However, only occasional reports of such material have appeared, and little is known about the nature of the crystals or their significance.

The earliest report of crystals in the thyroid gland appears to be that of Zeiss,<sup>1</sup> who mentioned, without further description or comment, the presence of octahedral crystals of calcium oxalate in the gland. Podack<sup>2</sup> mentioned various crystalline forms, among them calcium oxalate, and calcium or magnesium salts of a higher fatty acid.

Günther<sup>3</sup> described "crystalloids" as protein-like substances that are different from the crystals under consideration, but he also noted inorganic birefringent crystals of complicated form, soluble in hydrochloric acid. The latter, presumably, were of the type now under discussion.

Sanderson-Damberg<sup>4</sup> studied the thyroid glands of persons from 15 to 25 years of age, from different localities. She made the interesting observation that in those from Bern, in which there were small, uniform vesicles with vacuolated colloid, crystals of calcium oxalate were commonly found, while in thyroid glands from Kiel, Berlin, and Königsberg, in which the vesicles were large, no crystals were found.

Buscaino<sup>5,6</sup> studied the solubility of the crystals in various agents. He noted that they were dissolved by hydrochloric and nitric acids, and that sulfuric acid transformed them into an opaque mass. In acetic acid the crystals remained unaltered. Sodium hydroxide (40 per cent) and potassium hydroxide (50 per cent) also transformed them into granular masses that retained low-grade birefringence. The crystals were blackened by silver nitrate in the von Kossa reaction. On the basis of these reactions, Buscaino concluded that the crystals were probably calcium oxalate. He noted, however, that they differed in form from the ordinary (octahedral) crystals of that material. Buscaino's only statement regarding the relation of the crystals to disease was the suggestion that an increase occurred in cases of dementia praecox with epilepsy.

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One of us<sup>7</sup> has studied the occurrence of the crystals in 547 glands, and noted that they occurred in a greater proportion of the older than of the younger individuals, and in a greater proportion of normal than of abnormal glands in the same age group. It was suggested that the occurrence of the crystals is related to the functional state of the gland. Solubility studies gave results similar to those of Buscaino, except that solution occurred in dilute acetic acid. The crystals were not found in the thyroid glands of lower animals.

Martino<sup>8</sup> found the crystals in 22 of 30 cases of various ages. He observed that under the age of 30 years (7 cases) no crystals were found, while over that age they were present in 23 of 26 cases. They occurred in all (14) cases over the age of 50 years.

The crystals described by Popoff<sup>9</sup> and by Lichtman and McDonald<sup>10</sup> are of different nature.

#### MATERIAL AND METHODS

The preliminary studies of the senior author have been supplemented by observations of thyroid glands from 485 necropsies and a smaller number of surgically removed glands. Tissues were fixed routinely in Zenker's stock solution without addition of acetic acid, or in Zenker's-formol (Helly). The tissues were dehydrated in alcohol, embedded in paraffin, and the sections stained with hematoxylin and eosin.

Sections were examined with transmitted light, both diffuse and polarized. The presence or absence of crystals was noted by scanning the sections (often only one section of a gland) under a magnification of about 100 diameters, with crossed polarizer and analyzer. Inasmuch as crystals lying in certain directions to the plane of polarized light are invisible, as will be noted, this method may not disclose all of the crystals. Because of this fact, as well as the uneven distribution sometimes found, division of the glands into two groups, those in which crystals were found and those in which they were not, is somewhat arbitrary.

Crystals were isolated from fresh glands by placing scrapings or small fragments of tissue in physiologic saline solution, and teasing the tissue out under low magnification, with polarized light. This is most conveniently done with a binocular dissecting microscope. The crystals were removed from the colloid with dissecting needles, and placed on slides for further studies. For identification by x-ray diffraction techniques, crystals were placed in a thin-walled glass capillary tube, as will be described.

## OBSERVATIONS

*Description of the Anisotropic Material*

Examination of histologic sections between crossed polarizer and analyzer revealed, in certain instances and under the conditions to be described, minute particles of material that were brilliantly lighted against a dark ground (Fig. 2). The particles were of different sizes and of various forms: some were elongated polyhedrons; others were roughly spindle-shaped; still others were in the form of large irregular plaques. The most minute particles were barely visible with a magnification of 500 diameters. Small particles often were closely grouped but ordinarily had no special mutual orientation. Occasionally, however, radial arrangement of elongated particles was observed.

Considerably larger plaques often were cracked during sectioning. There also were many particles of irregular shape and of various sizes. As the object stage was rotated, extinction was observed at points  $90^\circ$  from the angle of maximum transmission.

The material was unstained and practically colorless, and thus was invisible or nearly so by ordinary examination in diffuse light (Figs. 1, 3, and 5). It was visible by dark-field examination which, however, illuminated only the edges of the particles. Occasionally they might be seen, but indistinctly, by partially closing the substage diaphragm. Once the particles were located in polarized light, their presence might sometimes be detected by diffuse light. Such examination, however, did not show the particles as clearly, nor in as large numbers, as with crossed prisms. Often the particles could not be seen in diffuse light even when their presence and position had been demonstrated with polarized light.

In both fresh preparations and in sections the particles might be moved, and the larger ones fragmented, by manipulation with a probe, thus indicating that they were material substances rather than refraction effects caused by varied molecular structure of the colloid.

With crossed prisms the particles usually were white, but suggestions of interference colors sometimes were seen, the color being faintly yellowish or purplish depending on the orientation of the particle with respect to the axis of polarization. On insertion of a retardation plate (first order red) these colors changed to deep yellow or red and blue, and were reversible on rotation of the object stage through  $90^\circ$ .

The foregoing description applies to the particles as seen in histologic sections of fixed thyroid glands, in which they occur only within the vesicles. They may also be seen in fresh teased glands, but the

presence of other anisotropic material renders observation difficult. The crystals do not occur in other organs.

### *Influence of Age*

The influence of age was observed in the preliminary studies of one of us<sup>7</sup> on glands from 443 necropsies from the Presbyterian Hospital and Babies Hospital. The increase was not uniform when studied by decades, but when the age groups were made larger, the increase of incidence with advancing age became apparent. This is shown in Table I, in which the 443 cases are divided into three groups accord-

TABLE I  
*Incidence of Crystals in Thyroid Glands in Different Age Groups*

Age groups	0-30 years			31-60 years			61-90 years			
	Posi- tive	Nega- tive	Posi- tive	Posi- tive	Nega- tive	Posi- tive	Posi- tive	Nega- tive	Posi- tive	
			%			%			%	
Presbyterian Hospital	15	70	17.6	93	139	40	79	47	62.7	443
University Hospital	8	92	8.0	106	121	46.7	86	72	54.4	485
Totals	23	162	12.4	199	260	43.3	165	119	58.1	928

ing to age. In the group up to and including the age of 30 years (85 cases), 15, or 17.6 per cent, contained crystals; in the age group from 31 to 60 years (232 cases), 93, or 40.0 per cent, had crystals; and over the age of 60 years (126 cases), crystals were found in 79, or 62.7 per cent. The probability that this result is due to chance distribution is less than 1 in 1000 (Chi-square = 85.9,  $N = 2$ ,  $P = < .001$ ).

A study of 485 glands from University Hospital (Table I) shows essentially the same distribution. In the cases from 0 to 30 years (100 cases), 8, or 8 per cent, contained crystals; from 31 to 60 years (227 cases), 106, or 46.7 per cent; and over 60 years (158 cases), 86, or 54.4 per cent. Again, the possibility of this being a chance distribution is less than 1 in 1000 (Chi-square = 176.8,  $N = 2$ ,  $P = < .001$ ). The combined Chi-square of the two series is thus 262.7,  $N = 4$ ,  $P = < .001$ .

### *Influence of Functional State of the Thyroid Gland*

Two observations clearly indicate that the occurrence of the crystals is related to the state of the gland. First, there usually was disappearance of the crystals in the diffuse hyperplasia of exophthalmic goiter, and in focal areas of hyperplasia. Second, in nodular goiters, there often were striking differences in the crystal content of different nodules. It was not unusual for some nodules to contain no crystals,

while in adjacent nodules, or in the surrounding zones, crystals were present in abundance (Figs. 4 and 6).

Forty-five additional thyroid glands, removed surgically for hyperthyroidism and showing diffuse hyperplasia, were examined. Crystals were found in only five glands, in four of which there was partial involution. The crystals found were in involuted areas and were few in number. In the fifth case, only a single small crystal was observed. Forty glands had no crystals. Several sections from each of the surgically removed glands were examined, so the results are not comparable to those from glands of necropsied cases. They do show, however, the comparative rarity of crystals in hyperplasia.

#### *Nature of the Anisotropic Crystals*

By immersing slides in various solvents, the following facts were observed. The material was readily soluble in dilute acids (hydrochloric, sulfuric, acetic). It was relatively insoluble in water (cold or hot), ethyl alcohol, acetone, chloroform, xylol, diethyl ether, petroleum ether, benzene, normal butyl alcohol, and glacial acetic acid. Crystals were still present after fixation in formalin or Zenker's solution without addition of acetic acid, but fixatives containing acid dissolved them. They remained unstained by hematoxylin and eosin.

The crystals could be dissolved from the fresh glands or from sections by dilute acids, but on re-crystallization by evaporation the minute crystals obtained were not readily recognizable as of the same nature as those originally present. Attempts to remove the colloid without dissolving the crystals usually resulted in a viscid mass that prevented purification of the crystals even on long centrifugation.

A number of single, large crystals were dissected from sections and placed in a drop of distilled water on a coverslip. The water was removed by allowing the coverslip to stand in a  $\text{CaCl}_2$  desiccator, after which the coverslip containing the material was placed in an incinerator. As the temperature was raised to  $300^\circ \text{C}$ . in 30 minutes, the crystals showed reduced birefringence. No change in shape of the crystals occurred on heating to  $700^\circ \text{C}$ . in 50 minutes. Similar observations were previously made by the senior author<sup>7</sup> by incinerating mounted sections.

Because of the presence of colloid around the crystals, it was believed that determination of their optical properties and histochemical reactions would be unreliable. Instead, the identification of isolated crystals by x-ray diffraction was attempted.\* Considerable difficulty

\* The x-ray diffraction studies were made by Dr. I. Fankuchen at the Polytechnic Institute of Brooklyn. We are indebted to him for his collaboration and for this description.



was encountered due to the fact that the crystals were embedded in colloid. At first, attempts were made to study the diffraction pattern of single perfect crystals, removed from sections by means of a metal dissecting needle and attached to the end of a glass fiber by using shellac. Photographs so made were unsatisfactory, as a conclusive pattern was not obtained. Therefore, thirty crystals were isolated and packed, without grinding, into one end of a thin-walled glass capillary tube. Grinding was avoided because of the danger of destroying the crystals. The capillary tube was mounted in the usual way on the arcs of a goniometer, and Debye-Scherrer (powder) diagrams were obtained. The radius of the cylindric camera was 5 cm. Filtered Cu radiation was used. The spacings and intensities of the lines on the x-ray patterns were then compared with those listed in the index of the American Society of Testing Materials. The compound was readily identified as calcium oxalate monohydrate.

#### DISCUSSION

Calcium is a normal constituent of cells and tissues, and also occurs in the form of pathologic calcifications and concretions. Crystals of calcium oxalate are commonly found in urinary sediments, but otherwise calcium compounds do not normally occur in crystalline form, with the exception of those in the colloid of the thyroid gland, as has been described. Oxalates, on the other hand, are toxic. They are eliminated as calcium oxalate in the urine, and are found in the renal tubules as calcium salts in oxalic acid poisoning. It is surprising, therefore, to find crystals of calcium oxalate occurring in the thyroid gland in a high proportion of all adults.

The significance of this finding will not be clear until the factors determining the formation of the crystals are known. An obvious factor in crystallization is the concentration of the substance in solution. Since histologic examination discloses only that portion of the calcium content that has crystallized, it cannot be used to indicate the total amount present. As the solubility product of calcium oxalate is small, crystallization may be expected in even very low concentrations of oxalate ion. There is no immediate explanation, however, for its occurrence in the thyroid gland.

Another factor is introduced by the occurrence of crystallization in a colloid rather than an aqueous medium. The presence of colloid not only may influence the formation of crystals, but may also alter their morphology. The possibility that the observations made are due to changes in thyroid colloid with age and disease, as well as to variations in the oxalate and calcium content of the gland, must be considered.

## SUMMARY

Anisotropic crystals occur in the colloid of the thyroid gland, but are not normally found in other organs.

There appears to be a tendency for the crystals to occur more often in older than in younger individuals.

The crystals are rarely found in exophthalmic goiter, and their occurrence in nodular goiters varies considerably. It is not uncommon to find nodules without crystals closely associated with other nodules in which crystals are abundant, or surrounded by crystal-containing vesicles of the more normal thyroid tissue. In other cases crystals appear within nodules, but not in the surrounding tissue. These findings indicate that the occurrence of the crystals is influenced by the state of the gland.

The anisotropic crystals have been identified by x-ray diffraction methods as calcium oxalate monohydrate.

It is suggested that the development and morphology of the crystals is influenced by the colloid, as well as by the concentration of calcium and oxalate ions in the secretion.

The photomicrographs were prepared by the staff of the Armed Forces Institute of Pathology.

## REFERENCES

1. Zeiss, O. Mikroskopische Untersuchungen über den Bau der Schilddrüse. Inaugural Dissertation, Strassburg, 1877, 50 pp.
2. Podack, M. Beitrag zur Histologie und Funktion der Schilddrüse. Inaugural Dissertation, Königsberg, 1892, 53 pp.
3. Günther, G. Über ein Krystalloid der menschlichen Schilddrüse. *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, 1896, 105, Abt. 3, 341-346.
4. Sanderson-Damberg, E. Die Schilddrüsen vom 15.—25. Lebensjahr aus der norddeutschen Ebene und Küstengegend, sowie aus Bern. *Frankfurt. Ztschr. f. Path.*, 1910-11, 6, 312-334.
5. Buscaino, V. M. La struttura della tiroide e le sue variazioni qualitative. *Riv. di pat. nerv.*, 1914, 19, 385-421, 449-498.
6. Buscaino, V. M. Ricerche sul significato biologico delle alterazioni qualitative della tiroide. I. Caratteri biochemici del siero di sangue di individui normali, di epilettici e di paralitici progressivi. *Riv. di pat. nerv.*, 1915, 20, 65-77. Ricerche sul significato biologico delle alterazioni qualitative della tiroide. II. Sulla spiccata eosinofilia del citoplasma in genere, nella tiroide di Basedowici in specie. *Ibid.*, 1915, 20, 152-156. Ricerche sul significato biologico delle alterazioni qualitative della tiroide. III. Cristalli ottaedrici di natura proteica ed epilessia. Accesso epilettico e crisi anafilattica. *Ibid.*, 1915, 20, 257-273.
7. Richter, M. N. Anisotropic crystalloids in the human thyroid gland. (Abstract.) *Am. J. Path.*, 1940, 16, 654-655.
8. Martino, L. Sulla presenza di microscopici cristalli nella ghiandola tiroide dell'uomo. *Boll. Soc. ital. biol. sper.*, 1942, 17, 486-487.

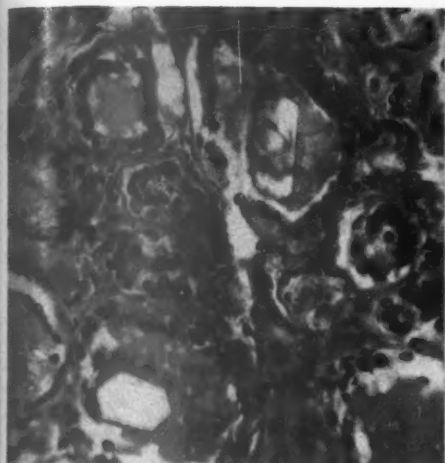
9. Popoff, N. W. Mechanism of release of colloid and the significance of the specific crystalline substance demonstrated in the thyroid gland histologically. *Arch. Path.*, 1943, 36, 587-601.
10. Lichtman, A. L., and McDonald, J. R. Birefringence in tissues. *Arch. Path.*, 1946, 42, 69-80.

#### LEGENDS FOR FIGURES

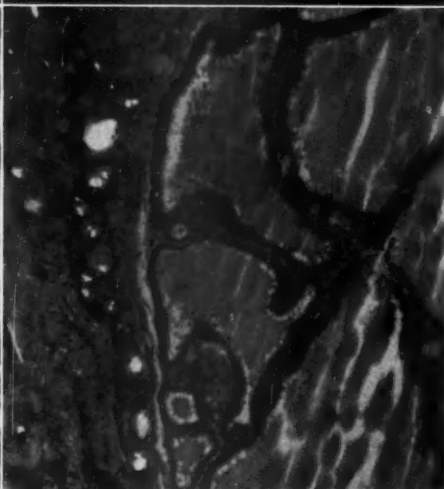
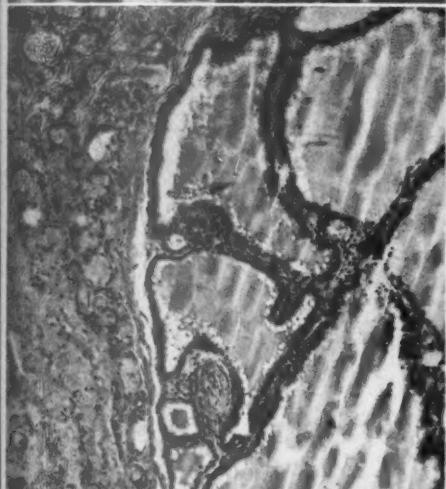
- FIG. 1. Section of thyroid gland, stained with hematoxylin and eosin, and photographed by diffuse light. The outlines of some of the crystals are seen in the acini.  $\times 300$ .
- FIG. 2. Same field as shown in Figure 1, but photographed by polarized light with crossed polarizer and analyzer. The crystals are more clearly seen than in diffuse light.  $\times 300$ .
- FIG. 3. Edge of nodule in thyroid gland, photographed by diffuse light.  $\times 100$ .
- FIG. 4. Same field as seen in Figure 3, but photographed by polarized light. The polarizer and analyzer are only partially crossed, so that details of the gland remain visible. Crystals are seen only in the zone around the nodule.  $\times 100$ .
- FIG. 5. Edge of nodule in thyroid gland. Photographed by diffuse light.  $\times 100$ .
- FIG. 6. Same field as shown in Figure 5, but photographed by polarized light with polarizer and analyzer partially crossed. Crystals are seen only within the nodule.  $\times 100$ .



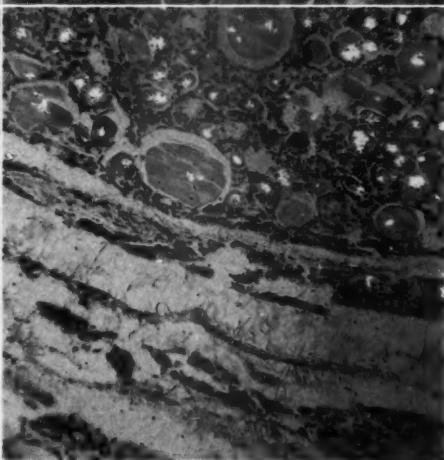
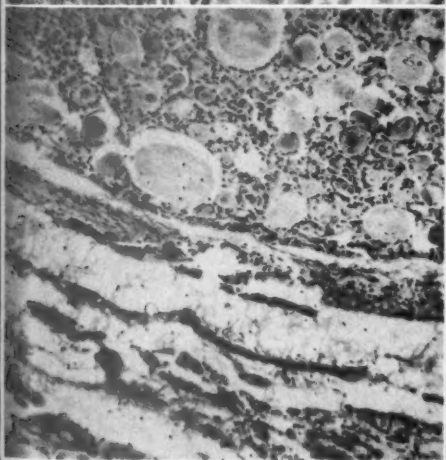




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SO-CALLED NUTRITIONAL MUSCULAR DYSTROPHY AS A CAUSE  
OF "PARALYSIS" IN RABBITS \*

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The disease known as nutritional muscular dystrophy, which occurs in laboratory animals, has a singular history. It was originally recorded as an experimental condition; whereas most diseases are first chronicled as an observed pathologic event, and then attempts are made by experimentalists to elucidate more about their nature and cause than can be deduced by the method of direct observation. The first description of this experimental morbid process, in guinea-pigs and rabbits, was made by Goettsch and Pappenheimer,<sup>1</sup> although it may have been seen as a natural disease in rabbits long before that period (Hobmaier<sup>2</sup>). A flood of papers appeared after 1931, some concerning its production in other species such as rats, mice, hamsters, ducklings, and tree kangaroos (Goss<sup>3</sup>), but little that is fundamentally new has been contributed to its pathology since the article by Goettsch and Pappenheimer. There were even more intensive studies in other directions, with some conflicting results, especially on the nutritional factors involved in etiology, and from thence emphasis has been on the causal rôle of alpha-tocopherol deficiency. (See, for example, Victor,<sup>4</sup> Mackenzie and McCollum,<sup>5</sup> Mackenzie *et al.*,<sup>6,7</sup> Madsen,<sup>8</sup> Morgulis *et al.*,<sup>9</sup> Pappenheimer,<sup>10</sup> Hove and Harris,<sup>11</sup> and Follis.<sup>12</sup>)

The name given to the disease may be unfortunate, as it may be perpetuated, but only Chor and Dolkart<sup>13</sup> have challenged this issue. As they intimated, the term muscular dystrophy immediately connotes some clinicopathologic resemblance to human forms classified under this terminology, which is quite erroneous. The label, in human neurologic medicine, is a generic one covering a group of different disorders—some purely functional—but in none of these is there any remote resemblance, in a pathologic sense, to the lesions seen in laboratory animals. (Unless such lesions do occur in human cases of non-paralytic "poliomyelitis" or pleurodynia (epidemic myalgia) from which the Coxsackie virus has been isolated.) Chor and Dolkart believed that myodegeneration is a more appropriate name, and that the lesions are more like those which have been described in typhoid fever in man. (In some older veterinary papers<sup>2</sup> the disease, commonly known as *weisses Fleisch* in domesticated animals, was referred to years ago as *myodegeneratio hyalinosa toxica calcificans*, or later

\* Received for publication, September 1, 1953.

simply as polymyositis.) Subsequently, the connotation was carried over to a cause, vitamin E deficiency, and from thence blossomed forth ideas on therapeutic treatment of human muscular dystrophies by tocopherols. According to some authorities, *e.g.*, Bicknell,<sup>14</sup> Spillane,<sup>15</sup> and Adams *et al.*,<sup>16</sup> the position is by no means clear, and possibly all claims resulting therefrom, regarding the efficacy of tocopherols in human muscular dystrophies, may have been somewhat premature. As Adams *et al.* pointed out, this is certainly so regarding amyotrophic lateral sclerosis or progressive muscular dystrophy. The term muscular dystrophy, for this disease in laboratory or domestic animals, should thus possibly be discarded; the lesions are, in essence, a scattered focal or disseminated type of myositis or degenerative myopathy, and apparently without any primary defect in the myoneural junctions (Rogers, Pappenheimer, and Goettsch,<sup>17</sup> Chor and Dolkart, and Adams *et al.*)

Although there is no clear analogy in human pathology to this disease of voluntary muscle of laboratory animals, it is important to mention the fact that there are parallel morbid entities in the domesticated species. In the latter, the condition referred to as *weisses Fleisch* (*cf.* Fig. 6) has a history, at least in German literature, dating back to 1886, a fact which seems to have eluded scrutiny by earlier investigators engaged in this problem of experimental nutritional pathology. In the fine treatise by Adams, Denny-Brown, and Pearson<sup>16</sup> there is unfortunately almost no reference to muscular disorders of domesticated animals, yet many valuable lessons might be obtained from comparative studies. The literature on myopathies in general in domesticated animals, together with an account of some peculiar cases, was reviewed by one of us.<sup>18</sup> For those medical workers interested in the comparative pathology of diseases of muscle in domesticated animals, useful reference might be made to Seifried<sup>19</sup> and Nieberle and Cohrs<sup>20</sup>; a paper by Hjärre and Lilleengen<sup>21</sup> on *weisses Fleisch* is comprehensive in original observations, pathologic description, and in bibliography.

#### THE DISEASE IN RABBITS

During the course of routine pathologic examinations on large numbers of experimental rabbits, which are constantly used in our laboratories for diverse toxicologic projects, a severe case of so-called nutritional muscular dystrophy was seen in one animal which had a history of progressive paralysis given to us by the contributing experimentalist. The technical procedure by the latter had involved only the use of a chemical compound, which had no deleterious effect on the

skin of the back after it was applied percutaneously. This meant that the deep-seated lesions in the intra-abdominal muscles, around the lumbar vertebrae, could have no etiologic connection with the experimental cutaneous treatment. The microscopic changes were of the classical type, primarily portrayed by Goettsch and Pappenheimer,<sup>1</sup> but were of devastating severity in the extent of the degeneration, necrosis, and concomitant inflammatory reaction. As the lesions specifically affected the psoas group on both sides, it was manifest that this was the anatomical basis for the reported paralysis and the gross wasting of the loins in the rabbit concerned. Thereafter, as part of routine necropsy work on rabbits, whether derived from normal stock or experimental colonies, particular attention was devoted to examination of the skeletal muscles, always including the paravertebral group. Several facts emerged therefrom, which might be of interest to those in charge of rabbit-breeding colonies, or to those working with large numbers of rabbits experimentally.

The word paralysis, when applied to laboratory animals, may be used by experimentalists in a haphazard way. It might, for instance, signify muscular weakness, rather than paralysis in a neurologic sense. Gross emaciating illnesses in rabbits, such as severe intestinal coccidiosis with its dysentery among others, may indeed result in a pseudo-paralytic syndrome because the animal may be too weak to move. In necropsy examinations on laboratory animals by the pathologist, as a service to some collaborator who has conducted technical experimental work on living animals, circumspection must be exercised in evaluating the significance of a clinical label of paralysis; this may obviate profitless examination of the central nervous system. Conversely, laboratory animals may show lesions in the central nervous system without specific neurologic signs, which has been commented upon by some who have studied experimental iso-allergic encephalitis (Lumsden,<sup>22</sup> Olitsky *et al.*,<sup>23</sup> and one of us, J. R. M. I.).

Since observing the first rabbit cases, more than 100 rabbits have been subjected to necropsy for various reasons. Of these, 24 animals showed this same type of damage to skeletal muscle, always affecting the same para-vertebral group. In a few rabbits this was accompanied by wasting of the loins, and thus by some impaired locomotor ability during life, but no true paralysis. In others, however, no clinical signs were detected denoting the presence of any muscular disease. When macroscopic lesions were present (in 16 rabbits) they were unmistakable, with their tangible hemorrhagic streaks intermingled irregularly with minute pallid spots and larger areas of virtual necrosis; in

the most severe types the lesions showed as friable patches of yellowish dead tissue. Of equal importance, however, was the fact that only microscopic lesions were found in one or both psoas muscles in 8 animals, which had been in plump healthy condition, and which had exhibited no cardinal weakness of the hind limbs. Very occasionally foci were found in heart muscle, but there were no cases with generalized disease involving many different anatomical locations.

The histologic changes were identical with those described years ago by Goettsch and Pappenheimer.<sup>1</sup> These structural alterations (Figs. 1 to 4) comprised waxy or hyaline degeneration, lumpy cleavage of some bundles of fibers with loss of striation, atrophy of other groups of fibers with reactive sarcolemmal multiplication, irregular confluent zones of hemorrhage and necrosis, and a concomitant inflammatory process in which all the cellular elements of the blood might be involved. Foci of pigment-bearing phagocytes occasionally were present; and in some cases, perhaps of longer duration, replacement fibrosis and calcareous deposition were prominent. However, we sometimes were impressed by a myolytic appearance of areas, like that following infections, in which the muscle fibers were stained a pale pink, striation being entirely lost, and in which even the sarcolemmal nuclei had disappeared. This latter appearance is duplicated to some extent in the muscle lesions of so-called paroxysmal paralytic myoglobinuria of horses.

In those animals in which only microscopic lesions were present, smaller foci, consisting of some of the above changes, were found, and the difference was thus only one of degree and not of kind of change. The very earliest tissue damage appeared to be swelling, ballooned vacuolation, and then a splitting of fibers, with some interstitial edema and hemorrhages. Sarcosporidia were found in many fibers of the sections examined, but not necessarily in those near or in the affected zones of muscle.

#### DISCUSSION

It would seem indisputable that the described lesions of a spontaneous disease in rabbits, precisely similar to that seen by one of us (J. R. M. I.) in rabbits in England (Fig. 5), are identical with those of the experimental, nutritional, deficiency condition, whether found in rabbits, guinea-pigs, rats, or mice. Despite the earlier controversy and claims that the disease might be a toxic effect of cod-liver oil, it is now commonly believed to be caused by an alpha-tocopherol deficiency in the diet. Apparently, white muscle of rabbits is more susceptible than the red variety, and as the disease progresses there is an absolute, as well as a relative, loss of creatinine (Goettsch and Brown<sup>24</sup>).

Whether the psoas and related vertebral muscles are particularly prone, or are a primary site of predilection, which may be the only location involved in a disease which occasionally may be generalized, we do not know. The restricted isolation of the process to this muscle group in many of our rabbits was a salient feature. Further, the inflammatory process in some animals had extended locally by contiguity, and had then involved the tendinous attachments, the adjacent fascia, and connective tissue.

We made no elaborate attempt to determine the cause. All rabbits in these laboratories had been purchased from commercial sources, and it had been the practice to feed the animals (stock and experimental) on one of the nationally known rabbit pellets, which was labelled as containing all requisite dietary constituents for rabbits. None of our rabbit cases was in an animal obtained only a few days before, which presumably would have meant origin of the disease before receipt. All grades of the disease, however, have been seen in both stock and experimental animals, and some of the latter had been kept on toxicity experiments of some type for many weeks or even months. Subsequently to the diagnosis of the condition, all rabbits have been given daily rations of roots and greenstuff, in addition to the pellets, and since then few other cases (none recently) have come to light. (Rabbits may, under some conditions, be reared on an entirely dry diet<sup>25</sup> although many with requisite experience believe that they can be bred, reared, and maintained better with additional greens and tubers.) Possibly the cause was therefore dietary, but our observations in this direction are practically uncontrolled and are immaterial to the main purpose of this article. No attempts were made by us to isolate an infective agent, bacterial or viral. We can omit consideration of sarcosporidial infestation as a cause. (See remarks by Innes.<sup>18</sup>) Adams *et al.*<sup>19</sup> made lengthy valuable statements on the comparison of the lesions in this myodegeneration with those in Coxsackie virus infection of suckling mice, and with those produced experimentally by plasmodium (a quinoline compound which is a protoplasmic poison) injected parenterally. They made pertinent comments on the non-specificity of the reaction of voluntary muscle to divergent causal factors.

Although bruising and damage of muscle fibers in the paravertebral group is known to be a possible sequel to rough handling of the rabbit, or to overvigorous mating, we do not consider that trauma *per se* could have been a cause in our series of rabbits.

This paper emphasizes the possible occurrence of an intercurrent disorder which may have been seen frequently by pathologists working with rabbits. There is no mention of the condition by Jaffé,<sup>26</sup> but



his treatise on diseases of laboratory animals had appeared before Goettsch and Pappenheimer<sup>1</sup> had published their first paper. However, the disease still has to achieve prominence by more than passing reference in books dealing with diseases of rabbits (Blount,<sup>27</sup> Worden,<sup>28</sup> and Seifried<sup>29</sup>).

This myositis may vary in severity from a clinically inapparent or silent disease with lesions which may be only microscopic, to a type in which the damage may be ravaging and no doubt very painful to the rabbit. Evidence of the more severe forms could materialize in all grades of motor weakness up to virtual paralysis of caudal movement. It is overtly a disease which might be coincidental to some experimental condition, and thus could easily cause erroneous clinical interpretation of pathologic effects, as it did in our first cases.

#### SUMMARY

An intercurrent disease of skeletal muscle in rabbits is described. The lesions are characterized by degenerative and necrotizing changes, which are the counterparts of those in so-called experimental nutritional muscular dystrophy. The process seemed to have some predilection for the paravertebral group of muscles. The name muscular dystrophy may be somewhat misleading, for the process is essentially that of degeneration, necrosis, and inflammation. The disease may cause wasting of the loins and thus locomotor difficulty, or it may be clinically inapparent with only microscopic lesions. The condition might thus be a source of error in interpretation of clinically labelled paralysis in rabbits following an experimental procedure.

The photographs were mainly the work of Mr. John Cuculis, Pathology Branch, Chemical Corps Medical Laboratories, Army Chemical Center, Maryland, to whom our thanks are due.

#### REFERENCES

1. Goettsch, M., and Pappenheimer, A. M. Nutritional muscular dystrophy in the guinea pig and rabbit. *J. Exper. Med.*, 1931, **54**, 145-165.
2. Hobmaier, M. Die sogenannte Haemoglobinuria enzootica des Pferdes und ihr verwandte Krankheiten unserer Haustiere. *Arch. f. wissensch. u. prakt. Thierh.*, 1926, **54**, 213-222. Über eine Myodegeneratio hyalinosa calcificans bei Lämmern, nebst Bemerkungen über Muskelverkalkungen bei Schwein und Pferd. *Ibid.*, 1925, **52**, 38-47.
3. Goss, L. J. Muscle dystrophy in tree kangaroos associated with feeding of cod liver oil and its response to alpha-tocopherol. *Zoologica*, 1940, **25**, 523-524.
4. Victor, J. Metabolic and irritability changes in nutritional myopathy of rabbits and ducks. *Am. J. Physiol.*, 1934, **108**, 229-236.

5. Mackenzie, C. G., and McCollum, E. V. The cure of nutritional muscular dystrophy in the rabbit by alpha-tocopherol and its effect on creatine metabolism. *J. Nutrition*, 1940, 19, 345-362.
6. Mackenzie, C. G., Levine, M. D., and McCollum, E. V. The prevention and cure of nutritional muscular dystrophy in the rabbit by alpha-tocopherol in the absence of a water-soluble factor. *J. Nutrition*, 1940, 20, 399-412.
7. Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V. Uncomplicated vitamin E deficiency in the rabbit and its relation to the toxicity of cod liver oil. *J. Nutrition*, 1941, 21, 225-234.
8. Madsen, L. L. The comparative effects of cod liver oil, cod liver oil concentrate, lard and cottonseed oil in a synthetic diet on the development of nutritional muscular dystrophy. *J. Nutrition*, 1936, 11, 471-493.
9. Morgulis, S., Wilder, V. M., and Eppstein, S. H. Further studies on dietary factors associated with nutritional muscle dystrophy. *J. Nutrition*, 1938, 16, 219-227.
10. Pappenheimer, A. M. The pathology of nutritional muscular dystrophy in young rats. *Am. J. Path.*, 1939, 15, 179-183.
11. Hove, E. L., and Harris, P. L. Relative activity of the tocopherols in curing muscular dystrophy in rabbits. *J. Nutrition*, 1947, 33, 95-106.
12. Follis, R. H. The Pathology of Nutritional Disease. Charles C Thomas, Springfield, Ill., 1948, p. 127.
13. Chor, H., and Dolkart, R. E. Experimental muscular dystrophy in the guinea pig. A nutritional myodegeneration. *Arch. Path.*, 1939, 27, 497-509.
14. Bicknell, F. Vitamin E in the treatment of muscular dystrophies and nervous diseases. *Lancet*, 1940, 1, 10-13.
15. Spillane, J. D. Nutritional Disorders of the Nervous System. Williams & Wilkins Co., Baltimore, 1947, 280 pp.
16. Adams, R. D., Denny-Brown, D., and Pearson, C. M. Diseases of Muscle. A Study in Pathology. Paul B. Hoeber, Inc., New York, 1953, 556 pp.
17. Rogers, W. M., Pappenheimer, A. M., and Goettsch, M. Nerve endings in nutritional muscular dystrophy in guinea pigs. *J. Exper. Med.*, 1931, 54, 167-169.
18. Innes, J. R. M. Myopathies in animals. *Brit. Vet. J.*, 1951, 107, 131-143.
19. Seifried, O. Vitamine und Vitaminmangelkrankheiten bei Haustieren. F. Enke, Stuttgart, 1943, pp. 111 and 166.
20. Nieberle, K., and Cohrs, P. Lehrbuch der speziellen pathologischen Anatomie der Haustiere. G. Fischer, Jena, 1949, ed. 3, p. 647 et seq.
21. Hjærre, A., and Lilleengen, K. Wachsartige Muskeldegeneration im Anschluss an C-Avitaminose bei Kälbern. Ein Beitrag zur Ätiologie und Pathogenese des sog. "weissen Fleisches" beim Kalbe. *Virchows Arch. f. path. Anat.*, 1936, 297, 565-593.
22. Lumsden, C. E. Fundamental problems in the pathology of multiple sclerosis and allied demyelinating diseases. *Brit. M. J.*, 1951, 1, 1035-1043.
23. Olitsky, P. K., Casals, J., and Tal, C. Relative susceptibility of various stocks of mice to experimental disseminated encephalomyelitis. *Proc. Soc. Exper. Biol. & Med.*, 1950, 75, 276-279.
24. Goettsch, M., and Brown, E. F. Muscle creatine in nutritional muscular dystrophy of the rabbit. *J. Biol. Chem.*, 1932, 97, 549-561.



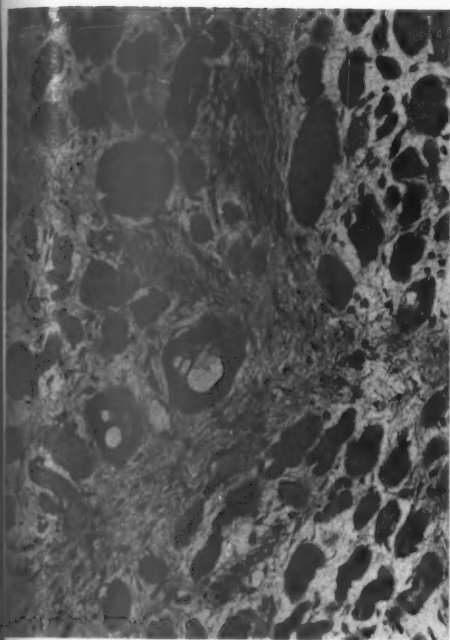
25. Bruce, H. M., and Parkes, A. S. Feeding and breeding of laboratory animals. II. Growth and maintenance of rabbits without fresh green food. *J. Hyg.*, 1945-46, 44, 501-507.
26. Jaffé, R. Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere (Kaninchen, Meerschweinchen, Ratte, Maus). Julius Springer, Berlin, 1931, 832 pp.
27. Blount, W. P. Rabbits' Ailments; a Short Treatise on the Domestic Rabbit in Health and Disease. Watmoughs Ltd., London, 1945.
28. Worden, A. N. (ed.) The Care and Management of Laboratory Animals. Baillière, Tindall & Cox, London, 1947, pp. 80-95.
29. Seifried, O. Die Krankheiten des Kaninchens mit besonderer Berücksichtigung der Infektions- und Invasionskrankheiten. Julius Springer, Berlin, 1937, ed. 2, 254 pp.

#### LEGENDS FOR FIGURES

- FIG. 1. Muscle of rabbit, showing swelling and ballooned vacuolation of certain fibers with some interfascicular edema. Hematoxylin and eosin stain.  $\times 127$ .
- FIG. 2. Muscle of rabbit. Another case showing atrophy of bundles of fibers with sarcolemmal proliferation and early fibrosis between bundles. Hematoxylin and eosin stain.  $\times 127$ .
- FIG. 3. Muscle of rabbit. A field showing hemorrhage and necrosis at the right and atrophy with cleavage, calcification of fibers, and fibrosis on the left. Hematoxylin and eosin stain.  $\times 127$ .
- FIG. 4. Muscle of rabbit, showing necrosis and myolysis of some fibers; early fibrosis; atrophy with sarcolemmal proliferation in other fibers. Hematoxylin and eosin stain.  $\times 127$ .



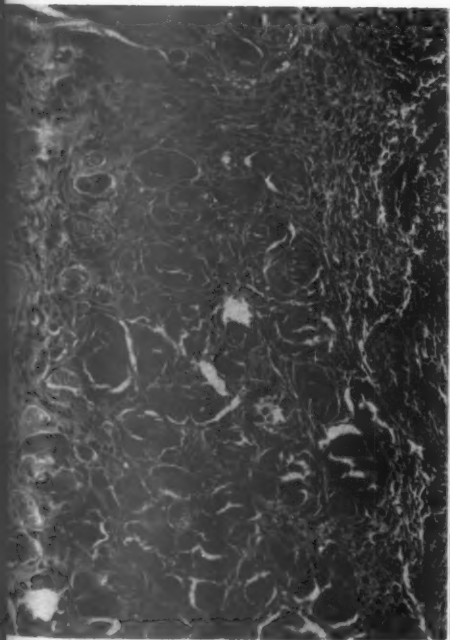




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FIG. 5. Muscle of rabbit. An example of spontaneous myodegeneration observed in England, showing necrosis, calcification, and inflammatory reaction. Hematoxylin and eosin stain.  $\times 130$ .

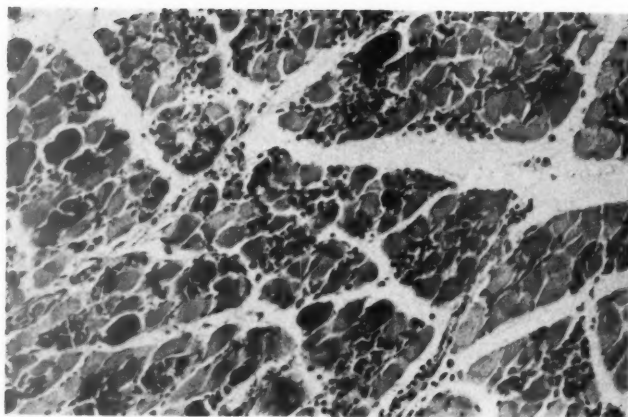
FIG. 6. Stiff-lamb, or white muscle disease (*weisse Fleisch*) of lambs, U.S.A., showing hyaline degeneration, necrosis, amorphous calcification of some fibers, inflammatory reaction, and early fibrosis. Hematoxylin and eosin stain.  $\times 130$ .



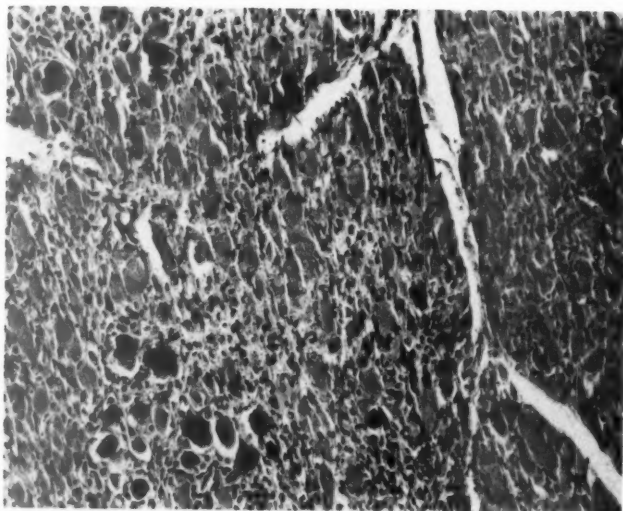




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PATHOLOGY OF TESCHEN DISEASE  
(VIRUS ENCEPHALOMYELITIS OF SWINE)\*

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Teschen disease, a viral encephalomyelitis of swine, was first described in 1930 by Treffny<sup>1</sup> during an epidemic in Teschen (Tessin), Czechoslovakia. It seems, however, that the disease was not unknown among veterinarians who, previous to this, had observed occasional sporadic cases of paralysis in pigs. Since 1930, it has been enzootic and frequently epizootic in Eastern Europe, but has never been known to occur in the United States.† Klobouk<sup>2</sup> first demonstrated its viral etiology in 1931, and during the next decade a number of pathologic and virologic studies were reported by Czech and German veterinarians.<sup>3-11</sup> These have been reviewed by Lépine<sup>12</sup> and Kaplan and Meranze.<sup>13</sup>

The present study of the pathology of Teschen disease was stimulated by frequent statements in the literature that a similarity existed between this disease and human poliomyelitis, both pathologic and otherwise.<sup>6-10</sup> Dobberstein,<sup>14</sup> who has reported the most extensive histopathologic investigations up to 1942, was of the opinion that the two diseases bear a close resemblance and he went so far as to speak of Teschen disease as "poliomyelitis of swine." A study of the pathology of Teschen disease was, therefore, undertaken with emphasis on the development and distribution of the lesions in the central nervous system. We also undertook to compare the pathologic changes with those of poliomyelitis and other viral encephalomyelitides, such as the equine, St. Louis, Japanese B, and louping-ill types. A preliminary report of the involvement of the central nervous system after intracerebral inoculation of Teschen virus has appeared<sup>15</sup> as has also a paper dealing with highlights of the pathology of the central nervous system in Teschen disease and a comparison with human poliomyelitis.<sup>16</sup>

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† For this reason, the importation of the virus into the United States is forbidden. All work with the agent, reported in this paper, was carried out in the Virus Laboratory of the 98th General Hospital, U.S. Army, Europe.

## MATERIALS AND METHODS

The material examined was obtained from some 300 pigs which were used during the course of a series of virologic studies on Teschen disease.<sup>17</sup> Several strains of virus\* were introduced intracerebrally, intranasally, and orally into pigs which were 4 to 6 weeks old.

The animals were sacrificed at different stages of the disease. Eleven were sacrificed as soon as they manifested an elevated temperature, which was interpreted as the earliest clinically detectable stage of the disease. The majority of the animals were killed at the height of the disease, which corresponded to the second or third day of fever, at a time when spasticity, convulsions, and often coma were present. If these animals had not been sacrificed, they would have died spontaneously within 24 hours. Since the mortality from the disease was extremely high, only 2 animals reached the convalescent period. They were sacrificed 1 month after the onset of the disease.

The distribution of the lesions in the central nervous system was determined in the following manner. Serial sections through a normal pig brain and spinal cord were made. An enlarged sketch was then made from each level of the central nervous system and a number of photographic copies of each sketch were made. Serial sections of the brain and spinal cord from each animal were examined, and concurrently the distribution of the lesions was plotted on the photographic sketch, which corresponded to the level of the microscopic section being studied. This was done on material secured from animals which were inoculated intranasally, intracerebrally, and orally. In this manner comparative distribution of the lesions in the various levels of the central nervous system was made evident.

The usual neuropathologic techniques were employed. The brain and spinal cord, and dorsal root, sympathetic, and gasserian ganglia of a number of animals were sectioned serially after embedding in celloidin.

## CLINICAL FEATURES

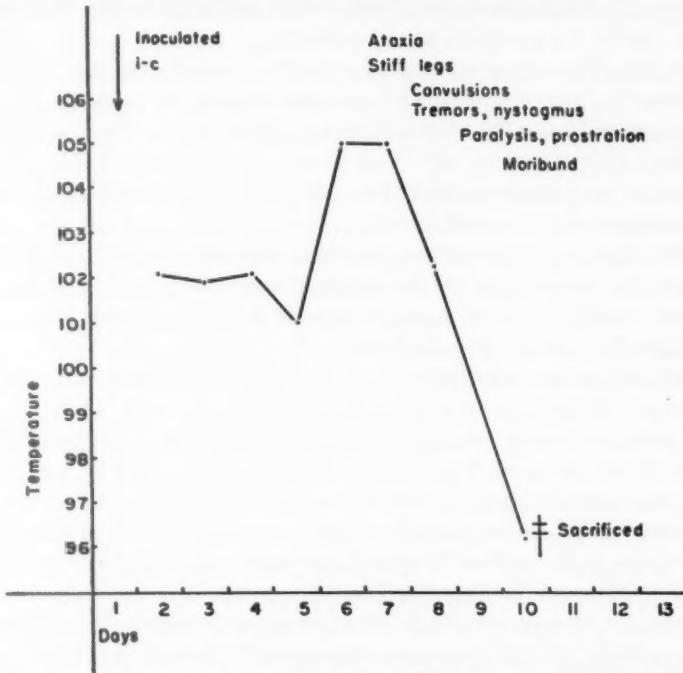
The disease in pigs, whether naturally occurring or experimentally induced, runs the course of a severe encephalomyelitis with a high mortality. The characteristic signs, in the order of their appearance, are fever, tremors, opisthotonos, stiffness of the extremities, ataxia, convulsions, paralysis of the cranial nerves, and weakness or paralysis of the extremities (Text-fig. 1). The incubation period varies with the

\* For these strains of Teschen disease virus we are indebted to the late Dr. Frantisek Gallia, National Institute of Health, Prague; to Dr. Sven Gard, State Bacteriological Institute, Stockholm; and Dr. Pierre Lépine, Pasteur Institute, Paris.

route of inoculation, but usually falls within 6 to 15 days.<sup>17</sup> Of many laboratory animals tested, the only susceptible hosts seem to be the pig and the wild boar.<sup>17,18</sup>

#### THE PATHOLOGIC CHANGES AND THEIR DEVELOPMENT

In many animals at the height of the disease the abdominal and thoracic organs revealed moderate to slight hyperemia. In some pigs there were tracheobronchitis, swelling of the spleen, and cloudy swelling of the liver. Occasional foci of pneumonia were noted.



Text-fig. 1. Course of Teschen disease in a 4-week-old pig inoculated intracerebrally.

The important morphologic manifestations of Teschen disease occur only in nervous tissue. Grossly, the brains of the animals killed at the height of the disease revealed edema of the meninges and congestion of the meningeal and cerebral vessels.

#### *Early Stages of the Disease*

Mild, focal, lymphocytic meningitis (Fig. 1) was a constant finding at the base of the brain. Polymorphonuclear leukocytes as the predominant cell type in the inflammation of the cerebral meninges were

seen in only one case. Also, moderate lymphocytic meningitis over the cerebellum was one of the earliest manifestations of Teschen disease. Over the cerebrum meningitis was less pronounced.

Mild lymphocytic infiltration was seen occasionally around small vessels of the first cerebral layer, as well as a few cell nodules, which occurred chiefly in the rhinencephalon. Moderate perivascular lymphocytic infiltration in the molecular layer of the cerebellum was a further early manifestation. In only 2 of the cases examined during the early stages of the disease were marked focal infiltrations composed predominantly of polymorphonuclear leukocytes seen in the molecular layer and in the meninges of the cerebellum.

At this time nerve cell changes in the cerebellar cortex, dentate nucleus, and roof nuclei either were not present, or were found only occasionally in the Purkinje cell layer and molecular layer and were minimal.

In the pons and medulla a few cell nodules and infiltrations were observed around the small vessels.

The *spinal cord* was without lesions in the majority of the cases. In 2 animals, in addition to the cerebral and cerebellar involvement, lesions were found in the anterior horn of the cervical cord, but not in the thoracic, lumbar, or sacral cord.

Only in one animal was found involvement of the anterior horns at all levels. In that case the destruction of the nerve cells (Figs. 2 to 6) was characterized by varying patterns of dissolution (chromatolysis) of the Nissl substance. The pattern of the Nissl substance became hazy and later dust-like, but at the periphery of some of the neurons and in the neuronal processes it remained well preserved. At this stage of the degeneration the nucleus frequently revealed slight hyperchromatism of its membrane and of the small dust-like particles in the nucleoplasm. This form of degeneration can occur also at the height of the disease. Degeneration of the cytoplasm might progress until the Nissl substance had completely disappeared. The cytoplasm of such cells was either basophilic or metachromatic to toluidine blue and might be swollen. Cytoplasmic vacuolization starting at the periphery and finally involving the whole cell body often was seen.

The anterior horn of the same animal in some sections was densely infiltrated with polymorphonuclear leukocytes which were actively phagocytizing dead nerve cells. In other levels of the cord, in addition to the degeneration of the nerve cells, a few neuronophagic and cell nodules and a moderate degree of perivascular infiltration were present. The microglia in the anterior horn showed marked proliferation.

The cells at first formed rod-like elongated or twisted elements (Fig. 7). However, especially in the early stages of the disease, marked degeneration of nerve cells accompanied by disproportionately moderate glial proliferation and slight perivascular infiltration might be seen in various levels of the spinal cord.

#### *Midcourse of the Disease*

*Spinal Cord.* Lesions were found localized in the anterior horns and in the posterior horns as well (Fig. 8). No differences in the involvement of the cervical, thoracic, lumbar, and sacral regions were observed.

One of the most characteristic features was the intense destruction of nerve cells and the presence of many neuronophagic and cell nodules in the cord. Often the cell nodules occurred in the neighborhood of vessels. Microglia were considered to be the predominating cells in neuronophagic and cell nodules; in these formations oval forms of these cells were the most frequent type encountered. Lymphocytes, in particular, always intermingled with microglial cells in areas of neuronophagia and in cell nodules. Many of the dead nerve cells were removed by phagocytosis (Fig. 9); others, especially those which were acidophilic in sections stained with hematoxylin and eosin, might occasionally undergo dissolution and be converted into a fluid-filled cavity.

Often the neuronal changes were more prominent in one half of the cord segment, while in the other half at the same level the proliferation of the glia or the perivascular infiltrations, or both, were prominent. The many mitotic figures were further evidence of the prolific activity of the microglia. Diffuse and nodular proliferation of microglia occurred also in areas where perivascular infiltrations were absent. The macroglia did not react as actively as the microglia. Hypertrophic astrocytes, however, were observed among the diffusely proliferating microglia and in the cell nodules.

There was a marked lymphocytic infiltration in the Virchow-Robin spaces of the involved areas. In addition to lymphocytes, a few plasma cells and histiocytes sometimes were seen. Lymphocytes and plasma cells might occur diffusely distributed in the anterior horn as well. Marked perivascular infiltrations were present in the white matter. Perivascular infiltrations occurred also in areas where neuronal damage was absent. The blood vessels were markedly congested. Very rarely, small hemorrhages occurred around the vessels.

The myelin of the intramedullary anterior root fibers was unaltered,



and gitter cells were not found. Bielschowsky staining revealed the neurofibrils to be well preserved in the early stages of neuronal damage. With progression of the degeneration of the nerve cells the neurofibrils of the cytoplasm became indistinguishable and finally disintegrated and disappeared.

Material fixed in Zenker's solution and formalin and stained with hematoxylin and eosin and Giemsa's stain was examined for inclusion bodies. Neither intranuclear nor cytoplasmic inclusions were present in the cord or in other regions of the central nervous system.

Perivascular infiltration and cell nodules also occurred in the posterior horn, although less frequently than in the anterior horn. Neuronal damage in the posterior horn was not found.

A slight lymphocytic meningitis usually was present in the diseased cervical, thoracic, lumbar, and sacral segments, and often its distribution was focal rather than diffuse.

*Medulla Oblongata.* Generally, the morphologic manifestations of the process at the level of the medulla oblongata were minimally different from those seen in the cord. The manifold degenerative forms of nerve cells, which were associated occasionally with slight glial and mesenchymal reaction, were not seen as often as in the cord, nor were areas of neuronophagia as prominent. On the other hand, perivascular cuffing and cell nodules were relatively more outstanding.

*Pons.* The lesions of the pons were quantitatively less pronounced than in the cord. There was not such a variety of neuronal degeneration nor were areas of neuronophagia so widespread. On the other hand, cell nodules and perivascular infiltrations prevailed here. The meningeal reaction was slight and consisted of a few lymphocytes and plasma cells.

*Cerebellum.* Marked changes occurred in the cerebellar cortex, especially in the Purkinje and molecular layers. The dentate nucleus and the roof nuclei also were involved. Meningitis was very marked in the cerebellum at the height of the disease. Dense infiltrations of lymphocytes and plasma cells were present, particularly in the sulci.

The Purkinje cells underwent considerable swelling and total vacuolization (Fig. 10); there was simultaneous retraction of the nuclear contents from the nuclear membrane, and the nucleus revealed granular hyperchromatism or a pastel-like basophilic discoloration. These nuclei soon perished. In the center of the pale vacuolated Purkinje cell one might find scant remnants of chromatin. Another change seen in the Purkinje cell was similar to Nissl's "akute Zellerkrankung," although the cell processes were not visible over long distances and the

outlines of the cell were not as distinct. Slight retraction of the cytoplasm from the surrounding tissue and of the nuclear contents from the nuclear membrane occasionally was present. Similar neuronal changes were seen in the molecular layer, although liquefaction of nerve cells was not as marked as in the Purkinje cells.

Large rows of Purkinje cells were destroyed (Fig. 11). Their former position was indicated by enormous proliferation of the microglia. Many "Strauchwerke" in the molecular layer indicated phagocytosis of the Purkinje cell processes. In many areas these formations fused into an extensive, diffuse, microglial proliferation. The bodies of the necrotic Purkinje cells were phagocytized by extensive nodular accumulations of microglial elements. Numerous areas of neuronophagia and of cell nodules were present (Fig. 12) in the molecular and Purkinje cell layer. In many instances it was difficult to distinguish between lesions of these two types, especially since the neuronophagic formations very often became confluent. Cell nodules of varying size also were present in the granular layer. Perivascular infiltrations were very marked, particularly in the molecular layer but also in the Purkinje cell layer.

*Mesencephalon.* Infiltrations and cell nodules were regularly found in the corpora quadrigemina. Usually perivascular infiltration was more severe in the substantia nigra than in the nucleus ruber; both nuclei, however, were involved at the height of the disease. In the majority of our cases the gray matter around the aqueduct revealed fewer lesions than the mesencephalic regions just mentioned.

*Diencephalon.* The diencephalon was regularly involved at the height of the disease. There were many foci of perivascular infiltration composed chiefly of lymphocytes, and cell nodules were very often found. Comparisons of the symmetrically cut thalami sometimes revealed marked loss of nerve cells on one side only. A large number of nerve cells in the thalamus underwent vacuolization of a type similar to that of the Purkinje cells. Vacuolization usually started at the periphery with retraction of the cytoplasm from the surrounding tissue. Neuronophagia of these dying cells was rarer in the thalamus than in the cerebellum. This was true also in regard to the glial reaction around these nerve cells. When complete vacuolization of many nerve cells occurred, large thalamic areas appeared perforated, the surrounding tissue might shrink and collapse, and occasionally cone-like microglial proliferations from the periphery of the cavity toward its center were seen. However, perivascular infiltration and cell nodules rather than the degeneration of the parenchyma dominated the

anatomical picture. Diffuse glial proliferations were not seen as often here as in the cerebellum.

Constant and marked manifestation of the disease was present in the subthalamus, the hypothalamus, and around the third ventricle. Here, as in the thalamus, perivascular infiltration and cell nodules prevailed; degeneration of nerve cells similar to the thalamic vacuolization was not observed in the hypothalamus.

Occasionally, a few perivascular infiltrations and cell nodules were present in the internal capsule.

*Globus Pallidus, Putamen, Caudate Nucleus, and Claustrum.* Infiltrations and cell nodules were found in the globus pallidus, putamen, caudate nucleus, and claustrum in decreasing order of severity. These nuclei were less severely involved than the diencephalon and mesencephalon.

*Cerebral Cortex.* The cortex revealed a moderately large number of loosely formed cell nodules and perivascular infiltrations (Fig. 13). These changes were most marked in the rhinencephalon. Definite regressive changes in nerve cells and areas of neuronophagia were rarely seen. In the cortex one might find shrunken nerve cells resembling the change described by Nissl as "chronic neuronal disease"; it is known that these shrunken neurons may occur in normal experimental animals.<sup>19</sup>

*Peripheral Ganglia.* The gasserian, cervical, stellate, thoracic sympathetic, and celiac ganglia of 34 animals, 3 of which were controls, were examined. At the height of the disease slight to massive infiltrations, composed chiefly of lymphocytes and less often of plasma cells, were found in the interstitium of the ganglia of 22 animals. These foci of infiltration, notably in the gasserian ganglion, were often nodular. In many cases the extent and severity of infiltration seemed to increase with longer survival after the onset of signs.

Foci of infiltration were marked in the gasserian, cervical, and celiac ganglia and were slight or absent in the stellate and thoracic sympathetic. In 3 of our 34 cases, the gasserian and cervical ganglia showed marked participation of polymorphonuclear leukocytes in the inflammatory process. In another case there were more plasma cells than usual.

Marked degenerative changes occurred occasionally in the neurons of the ganglia in Teschen disease. They were found most often in the gasserian, less so in the cervical, and rarely in the celiac ganglion. Definite neuronal degeneration was not seen in the thoracic sympathetic or stellate ganglia. The Nissl substance of the degenerated nerve

cells was dust-like and was concentrated at the periphery of the cell; often the perinuclear parts were slightly metachromatic. The nucleus of these chromatolytic nerve cells either was swollen or shrunken; the nucleolus usually was swollen. In later stages of degeneration the outlines of nucleus and protoplasm became indistinct. In addition to the neuronal degeneration, one saw early changes in the amphicytes of the capsule. These increased in number and size. By proliferation, they replaced the dying nerve cells until the whole capsule was filled with progressive amphicytes which often were mixed with proliferating connective tissue elements and lymphocytes. These proliferating cells might later decrease in number leaving a shrunken formation, the so-called residual nodule. Extremely rarely, neuronophagia of dead nerve cells by amphicytes was seen.

The spinal ganglia revealed degenerative neuronal changes and lymphocytic infiltration similar to those already described in the peripheral ganglia.

#### *Convalescent Stage of the Disease*

Corresponding to the findings in the acute stages of the disease were the changes seen in the convalescent animals. Two animals which survived showed cerebellar atrophy (Fig. 14), which in many folia was restricted to the Purkinje-cell layer. In other areas, however, many nerve cells in all three cerebellar layers had disappeared and a marked isomorphic gliosis was present. The external form and structure of the cerebellum were preserved. The molecular layer was decreased, the Purkinje cells were missing over large areas, and the granular layer was diminished. The Bergmann glia became multilayered and took the place of the Purkinje layer. In the atrophic cerebellar cortex there was a selective involvement of the layers, the Purkinje cells being the most vulnerable, followed in order of susceptibility by the molecular and finally by the granular layer.

The spinal cord revealed noticeable disappearance of nerve cells in the anterior horns. In such areas a few lymphocytes and plasma cells might be present around the small vessels in addition to the many astrocytes and microglial cells.

A comparative examination of the symmetrically cut thalami revealed a diminution of nerve cells in the more severely involved hemisphere. In one case two foci of perivascular infiltration were seen in the pulvinar.

In other regions of the central nervous system neither perivascular infiltrations nor cell nodules were present.

## DISTRIBUTION OF LESIONS

It is a characteristic feature of the brain in encephalitides that the reactive responses of the glia and the vascular mesenchymal tissue are quantitatively limited, and it is not uncommon for different agents to produce similar morphologic changes. In addition, alterations of nerve cells are frequently non-specific and usually do not assist in differential diagnosis of the encephalitides. Therefore, proper study of an encephalitic process requires consideration not only of the nature of the lesions but also of their distribution in the central nervous system. The distribution of the lesions (perivascular and parenchymal lesions, neuronophagic nodules, and cell nodules) during the early stage and during the height of Teschen disease will now be described.

Comparing the pig brain with that of other mammals, we attempted to locate the important regions of the porcine central nervous system. Figures 16 to 38 present drawings of a few serial sections of the pig brain and cord. The left half of each drawing shows the normal structure with the important regions depicted. The right half is marked with dots to give a composite representation of the lesions occurring in each area.

In the preceding discussion, the regions in the central nervous system described in detail represent, with the exception of the telencephalon, the most involved areas in Teschen disease at its height. The widespread distribution of the lesions at the height of Teschen disease is indicated in Figure 15. The most widespread involvement was present in the spinal cord and invariably in the cerebellum. Next most severely involved were the thalamus, medulla, and pons, followed by the mesencephalon. The nucleus olivaris was markedly involved in the majority of cases. Moderate perivascular infiltrations occurred consistently around the aqueduct.

Selective damage of bulbar and pontine nuclei was not observed; individual differences, however, might occur from case to case. In some animals, for instance, the basal parts of the pons were slightly more involved than the dorsal.

However, the over-all impression gained during study of the medulla and pons was that the dorsal and ventral portions of each were involved to approximately the same degree. To confirm this impression total lesion counts were carried out in the serial sections of the medulla and pons of 8 animals (3 intracerebrally inoculated, 3 orally, and 2 intranasally). These results are given in Table I. Marked variation among individual sections occurred; sometimes the ventral lesions outnumbered the dorsal or vice versa. This variation is indicated in the column maximum ratio in Table I which gives the numerical ratio

(dorsal/ventral or ventral/dorsal) for the individual section showing the most variation within a single case. When the total lesions occurring in all sections of a single case were considered, however, it was seen that there was no significant difference between dorsal and ventral involvement, at least in the 8 animals studied.

Involved with decreasing severity were the nuclei septi pellucidi; the pallidum and putamen; and, finally, the caudate nucleus and

TABLE I

*Relation of Distribution of Lesions in the Ventral and Dorsal Parts of the Medulla and Pons*

Case number and route of inoculation	Medulla			Pons		
	Number of lesions counted	Percentage	Maximum ratio	Number of lesions counted	Percentage	Maximum ratio
		V* D†	V/D D/V		V D	V/D D/V
12 Intracerebral	163	52 48	$\frac{3.0}{2.0} \frac{3.3}{2.7}$	581	54 46	$\frac{3.5}{1.8} \frac{3.5}{1.2}$
3 Intracerebral	133	52 48	$\frac{1.7}{0.8} \frac{2.2}{1.8}$	446	41 59	$\frac{1.6}{0.7} \frac{1.7}{0.3}$
18 Intracerebral	203	48 52	$\frac{4.3}{4.0} \frac{6.5}{5.5}$	506	52 48	$\frac{5.9}{2.4} \frac{5.7}{3.5}$
35 Oral	121	46 54	$\frac{0}{0} \frac{1.4}{1.0}$	158	32 68	$\frac{0}{0} \frac{2.4}{0.6}$
123 Oral	112	50 50	$\frac{1.2}{1.0} \frac{1.8}{1.6}$	182	57 43	$\frac{2.9}{1.5} \frac{8.0}{5.0}$
117 Oral	225	51 49	$\frac{3.1}{2.9} \frac{3.0}{2.7}$	437	50 50	$\frac{5.5}{4.7} \frac{5.6}{4.1}$
52 Intranasal	179	56 44	$\frac{0.9}{0.6} \frac{1.2}{0.7}$	180	52 48	$\frac{2.2}{1.2} \frac{2.3}{1.2}$
53 Intranasal	116	50 50	$\frac{2.5}{2.0} \frac{2.8}{2.3}$	145	61 39	$\frac{3.3}{2.1} \frac{0}{0}$

\*V = ventral.

†D = dorsal.

rhinencephalon. The remainder of the cerebral cortex revealed moderate changes, the base being more involved than the convexity (including the motor cortex). The frontal lobe and the basal temporal gyri were more affected than the parietal lobe. The occipital lobe was occasionally and mildly involved. The ependyma revealed no changes.

The white matter rarely disclosed perivascular infiltrations and cell nodules. Infiltration of the choroid plexus was seen in only one case.

In the incubation period, loose cell nodules and mild perivascular infiltrations were scattered at random in the cerebellum, rhinencephalon, and rarely in the pons and medulla. At that time the spinal cord usually was not involved.

Orally, intranasally, and intracerebrally inoculated animals were



compared with regard to the distribution of the lesions in the central nervous system. Taking into consideration that individual differences may occur from case to case among similarly inoculated animals, no characteristic differences were found in the distribution of the lesions in the early stages or in the midcourse of the disease among the three inoculated groups.

#### COMMENT

Spielmeyer<sup>20</sup> classified the encephalitides on the basis of the histologic nature of the inflammatory reaction. Considering the predominantly mesenchymal reaction in general paresis and the almost pure glial inflammatory response in typhus encephalitis as the two extremes of a scale, he called poliomyelitis a glial-mesenchymal inflammation. Similarly a glial-mesenchymal inflammation is seen in Teschen disease. The chief participants in the inflammatory reaction are lymphocytes and microglia. Predominance of polymorphonuclear leukocytes occurs only rarely and for a limited time in the early stages of Teschen disease.

Teschen disease is a non-purulent encephalomyelitis of the gray matter, the involvement of the white matter being rare and minimal. The destruction of nerve cells in some regions of the central nervous system, such as the spinal cord, is very rapid and severe. In other areas, such as the cerebral cortex, definite regressive changes in nerve cells are rarely seen and they seem to bear no definite relationship to either the number or extent of the foci of perivascular infiltration. Our findings are in agreement with those of Kment,<sup>8</sup> who did not find inclusion bodies; on the other hand Scheuer<sup>7</sup> reported "many inclusions" in the nerve cells.

Besides the degeneration of the nerve cells and the perivascular infiltrations, the cell nodules are among the characteristic morphologic features of Teschen disease. Although often occurring in close relation to the vessels, they may appear independently. These nodules may be either loosely formed or very compact and large. The former type predominates in the cerebral cortex while the latter occurs more frequently in the cerebellum. Glial cuffs around infiltrated vessels usually do not occur. Scholz<sup>21</sup> made the same observation in von Economo's disease. Sometimes there is a moderate, diffuse increase of the glia in the subcortical areas of the attacked cortex. Aside from these findings the white matter contains occasional perivascular infiltrations and, more rarely, cell nodules.

Although the more massive involvement occurs chiefly in the diencephalon, midbrain, cerebellum, medulla, and cord, there is virtually



no part of the brain that is not involved at one time or another. Compared with the severe morphologic manifestations in the regions just mentioned, the pathologic changes in the telencephalon are constant but moderate, revealing quantitative variations from case to case. The rhinencephalon and generally the base of the brain are more involved than the lateral parts and the convexity.

The brain in orally, intranasally, and intracerebrally inoculated animals is involved earlier in the course of the disease than is the spinal cord. This is clearly shown in the early stages of the disease, when fever is the only sign; then one may find, with lesions in the cerebrum and cerebellum, either no changes in the cord or involvement only of the cervical cord, the thoracic and lumbar regions remaining free.

There are differences of opinion among authors concerning the distribution of the lesions in Teschen disease. It has already been mentioned that many have been impressed by the localization of the process in the spinal cord and have emphasized this aspect. In the early literature there is only one report of a case with encephalitic involvement without changes in the cord.<sup>11</sup> Baumann,<sup>9</sup> Kment,<sup>8</sup> and others have reported that, next to the cord, the most severely involved areas are medulla and pons. In the cerebellum a varying leptomeningitis is said to occur.<sup>8</sup> Traub<sup>11</sup> stated that the cerebellum and thalamus are less involved than the olfactory bulbs and Ammon's horn and are as severely attacked as the striatum. All authors have found lesions in the striatum and pallidum, but there is disagreement concerning the severity of the involvement. Many have found lesions of moderate or slight degree in the cerebral cortex.

Recently Környey and Elek<sup>22</sup> found no changes in the striopallidum. Furthermore, they did not seem to be impressed by the moderate cerebral cortical involvement in their cases. Thus they classified Teschen disease with the "focal polioencephalitides with a predilection for the brain stem (Encephalitis epidemica type)."<sup>23</sup> Our experiences, in examining over 300 pig brains, differ in certain respects from the above statements, as has been pointed out in the preceding description of the lesions. Presentation of our convictions will be further clarified in the subsequent comparison of Teschen disease with other viral encephalitides.

The pathologic findings in several important areas of the central nervous system indicate a certain variability in the morphologic manifestations from region to region. There are, for example, marked and manifold degenerative changes in the nerve cells of the anterior horn. On the other hand, one must search long and hard to find regressive

neuronal forms in the cerebral cortex. In both the cerebellum and the thalamus nerve cells may undergo total vacuolization, but phagocytosis and glial reaction are different in the two regions. The morphologic manifestation appears to depend in some way upon the area involved.

The following can be stated concerning the relationship of "neuronal damage" to the "glial-mesenchymal reaction" in Teschen disease. Although the gliomesenchymal reaction in some regions is less extensive than the degeneration of nerve cells, we never observed isolated degeneration of nerve cells without reaction of the glia or mesenchyma even in the early stages of the disease. This does not mean, however, that the two reactions are necessarily and always interdependent, since perivascular cuffs may be present in areas without neuronal damage and one can see well preserved nerve cells among lymphocytes, plasma cells, and microglia, a finding which is known to occur in various encephalitides.<sup>24</sup>

More cells participate in the phagocytosis of nerve cells than appear necessary from a functional viewpoint. For instance the individual neuronophagic nodules and "Strauchwerk" formations in the cerebellum are much more cellular than the corresponding formations seen in non-infectious diseases. Thus it appears that the agent causing the disease is capable not only of inducing a primary glial-mesenchymal inflammatory response, but also of influencing the ultimate form of neuronophagia and "Strauchwerke."

Meningitis in Teschen disease is not always related and proportional to cortical damage. Meningeal reaction is a very early sign of the illness and occurs in the late incubation period either with or without minimal degeneration of the cortical nerve cells.

Care must be exercised in the interpretation of the findings in the ganglia since some of the so-called pathologic changes occur normally in the ganglia of man and animals. It has been reported that lymphocytes and mast cells occur under normal conditions in the ganglia of humans. For example, the inflammatory infiltration described in the stellate ganglia in causalgia was later found in cases unrelated to causalgia, such as accidental death.<sup>25</sup> Bodian and Howe<sup>26</sup> and Faber *et al.*,<sup>27</sup> working with poliomyelitis in monkeys, found changes in the ganglia in two of three control animals.

In selecting criteria of neuronal degeneration it is well to remember that the Nissl substance of nerve cells in the ganglia is normally not distinct and is often dust-like in appearance. There is individual variation in the number of cells present, and so-called "shrunk" nerve cells occur normally in ganglia. Neurons showing retraction of the

cytoplasm from the capsule and resembling "celulas fenestradas" (Cajal) also occur normally in the ganglia of the pigs.

The ganglia of many infected animals, especially in the early stages of Teschen disease, reveal mild inflammatory changes which do not differ either qualitatively or quantitatively from those seen in the controls. However, the ganglia of the more severely affected animals often contained more massive inflammatory changes and occasional neuronal changes as well. These latter alterations were never seen in the control animals.

The liquefaction and retraction of altered nerve cells is usually accompanied by proliferation of the capsule cells, neuronophagia occurring very rarely in ganglia. It is emphasized that these two processes are entirely separate.

Among the reports in the literature only Kment<sup>8</sup> mentioned liquefaction and shrinkage of nerve cells in the spinal ganglia as occurring in Teschen disease. In addition, he found infiltration composed of lymphocytes and macrophages. All of these findings were considered by Baumann<sup>9</sup> as non-specific for Teschen disease.

#### *Correlation of the Clinical Signs with the Anatomical Changes*

In encephalitis as widespread as Teschen disease it is difficult to localize the basis for individual neurologic signs. It cannot be proved, for instance, that the disturbances of coordination and equilibrium are due only to the midbrain involvement<sup>22</sup> or that the tonic convulsions and the opisthotonos are due to the lesions of the brain stem.<sup>22</sup> Since the neurophysiology of the pig is an uncharted field, it is dangerous to make restricted correlations between clinical signs and anatomical lesions. In general terms, however, one may correlate the severe involvement of the cerebellum and basal nuclei with the clinical manifestations of ataxia, forced movement, and tremors. In addition, the relatively late onset of paralysis of the extremities is in accord with the anatomical findings of late involvement of the spinal cord in this disease.

#### *Comparison of Teschen Disease with Other Viral Encephalitides*

On the basis of a single morphologic feature one may find resemblances among viral encephalitides. The perivascular infiltrates, cell nodules, and generally the glial response are similar in many viral inflammatory processes of the central nervous system, for instance, in Teschen disease, poliomyelitis in man and monkey, and Japanese B encephalitis in man.

Környey and Elek<sup>22</sup> believed that Teschen disease and von Economo's disease have striking morphologic similarities. In both, the involvement of the brain stem is very marked. However, in von Economo's disease the thalamus, a region of predilection in Teschen disease, is spared of lesions with the exception of a small paraventricular area.<sup>28</sup> Conversely, the cerebellar cortex, which in Teschen disease is constantly involved, is only "minimally or not at all altered" in epidemic encephalitis.<sup>28</sup>

Other authors have considered poliomyelitis and Teschen disease to be similar on the basis of changes occurring in the spinal cord. The changes are, indeed, strikingly similar; however, the involvement of the entire cord, and especially of the posterior horns, is more extensive in Teschen disease, and there is not the relative sparing of the thoracic cord that is frequently seen in poliomyelitis. Furthermore, the encephalitic manifestations of the two diseases are quite different. The motor cortex, a region of selective involvement in poliomyelitis, is only slightly altered in Teschen disease and appears to be much less vulnerable than other areas, *e.g.*, cerebellum, thalamus, midbrain, caudate nucleus, and rhinencephalon. More extensively involved than the motor cortex is the base of the brain, as has been mentioned.

The regions rarely if ever affected in poliomyelitis, according to Bodian,<sup>29</sup> "include primarily the entire cerebral cortex, except for the motor area, the corpus striatum, except occasionally for the globus pallidus, the cerebellar cortex except for the vermis, and the base of the pons." In Teschen disease these regions are constantly affected and their involvement is far more massive than in poliomyelitis. Polymorphonuclear leukocytes, which occur relatively often in the early stages of poliomyelitis, are rarely seen in Teschen disease. It is possible, of course, that they occur, but more transiently than in poliomyelitis.

Wolf published a useful classification of some common viral encephalitides grouped on the basis of the selectively affected regions.<sup>30</sup> He separated the encephalitides into three groups. In group one, which includes lethargic encephalitis, "one end of the brain, its caudal extremity, the brain stem, suffers most." In group three, including herpes simplex and inclusion encephalitis, "the rostral extremity of the brain, represented by the cerebral cortex, suffers most." The second group is composed chiefly of the arthropod-borne encephalitides. "In these, the lesions are found in all parts of the nervous system, with the brunt of the attack being borne by the basal structure of the brain and by the cerebral cortex."

Of the encephalitides included in group two, the equine encephalitides and St. Louis encephalitis reveal morphologic features entirely absent in Teschen disease; *e.g.*, involvement of both gray and white matter and areas of encephalomalacia.

There are many similarities between the distribution of the lesions seen in Teschen disease and those found in Japanese encephalitis in man.<sup>31</sup> Diencephalon, midbrain, cerebellum, and medulla are markedly affected. Cell nodules are characteristic in both, but in Japanese B, their distribution is more uniform and extensive in the cerebral cortex, especially along the lateral fissure and the upper convex surface of the brain, areas which are less involved in Teschen disease. Leptomeningitis in Japanese B is more striking over the cerebrum and minimal over the cerebellum, whereas the opposite is found in Teschen disease. Zimmerman<sup>32</sup> described circumscribed zones of acute degeneration, pale in appearance and without mesenchymal or glial response, in Japanese B encephalitis. Such degeneration does not occur at all in Teschen disease. Of the viral encephalitides, Japanese B, except for the differences mentioned, bears a close resemblance to Teschen disease.

There are many morphologic similarities between Teschen disease and louping ill, at least in experimental infections of monkeys<sup>33</sup> and pigs.<sup>34</sup> In both diseases, the cerebellum is markedly involved although the microglial reaction of the molecular layer in louping ill of monkeys is less pronounced. In louping ill, involvement of the cerebral cortex and caudate and lenticular nuclei does not show the quantitative differences seen in Teschen disease, nor is the involvement of the diencephalon so constant. In the cord acute necrosis is missing and changes ending in death of nerve cells are uncommon.

When the morphologic features are considered as an entity, Teschen disease is seen to differ from other viral encephalitides.

#### SUMMARY

A study of the pathology of Teschen disease reveals it to be a widespread encephalomyelitis confined almost entirely to the gray matter. The lesions consist of degenerative neuronal changes, perivascular infiltrations, and neuronophagic and cell nodules; the latter may be of loose or compact organization, are often found in relation to vessels, and occur in large number at the height of the disease. Microglial cells are the common elements of neuronophagic and cell nodules. The perivascular infiltrations are composed predominantly of lymphocytes.

The relationship of the neuronal damage to the reaction of glial and

vascular-mesenchymal tissue has been discussed. It was found that the perivascular infiltrations and the glial reaction can occur not only as a secondary response to neuronal degeneration but also as primary phenomena.

Meningitis is one of the early manifestations of Teschen disease; the inflammation of the meninges is very marked in the cerebellum, moderate in the cerebrum, and slight in the spinal cord.

The morphologic manifestations of the disease vary in intensity and quality in different parts of the central nervous system. The distribution of lesions in the early stages and at the height of disease has been discussed. In the fully developed disease the most severely involved areas are in the cerebellum, cord, thalamus, medulla, midbrain, and pons; less severely affected are the caudate nucleus, putamen, and pallidum. The cerebral cortical lesions are moderate and are more prominent in the rhinencephalon than in other parts of the cortex.

The findings in the peripheral ganglia in Teschen disease were studied and critically considered.

The morphologic changes in animals which had recovered from the acute stage of Teschen disease have been described.

Teschen disease has been compared with other viral encephalitides. When the comparison is limited to one or a few morphologic features, similarities to certain of these encephalitides are found. However, when the entirety of morphologic changes, including their quality, quantity, and distribution, is considered, it is not difficult to differentiate this disease from other viral encephalitides and to establish it as a separate disease entity.

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#### REFERENCES

1. Treffny, L. Massenerkrankungen von Schweinen in Teschner Land. *Zvěrolék. Obsor*, 1930, **23**, 235-236.
2. Klobouk, A. Encephalomyelitis enzootica suum (eine klinische und pathologisch-anatomische Studie). *Zvěrolék. Obsor*, 1931, **24**, 436-444, 460-464, 477-480.
3. Klobouk, A. Encephalomyelitis enzootica suum (ein durch Berkefeldsche Filter durchgehender ultravisibler Virus als Krankheitserreger). *Zvěrolék. Rosp-ravy*, 1933, **7**, 133-139, 157-167, 169-180, 181-188, 193-201, 205-208, 217-221, 229-234, 246-250, 253-257, 268-269.
4. Weidlich, N. Über die Teschener Krankheit (Encephalomyelitis enzootica suum). *Berl. u. Münch. tierärztl. Wchnschr.*, 1939, 549-552.
5. Ruml, L. Auftreten der Encephalomyelitis enzootica suum in Oesterreich. *Wien. tierärztl. Wchnschr.*, 1939, **26**, 1-6.



6. Frauchiger, E., and Hofmann, W. Die epidemische Kinderlähmung und die Teschener Krankheit der Schweine. *Schweiz. med. Wchnschr.*, 1941, 22, 584-585.
7. Scheuer, F. Altérations inflammatoires du système nerveux central dans la paralysie infectieuse du porc. *Bull. Inst. Pasteur*, 1937, 35, 629.
8. Kment, A. Zur Histopathologie des Zentralnervensystems bei der Teschener Krankheit. *Wien. tierärztl. Wchnschr.*, 1940, 27, 361-362.
9. Baumann, R. Die histologische Diagnose der Teschener Schweinelähmung (Encephalomyelitis non purulenta enzootica suum, sog. Böhmisches Seuche). *Berl. u. Münch. tierärztl. Wchnschr.*, 1940, 217-219.
10. Grau, H. Zur Diagnosestellung bei ansteckender Schweinelähme in den Veterinäruntersuchungsämtern. *Berl. u. Münch. tierärztl. Wchnschr.*, 1941, 85-88.
11. Traub, E. Aktive Immunisierung gegen die ansteckende Schweinelähme mit Adsorbatimpfstoffen. *Arch. f. wissenschaftl. u. prakt. Tierh.*, 1941, 77, 52-66.
12. Lépine, P. Encephalomyelitis enzootique des porcs. In: Levaditi, C., Lépine, P., and Verge, J. (eds.). *Les ultravirus des maladies animales*. Librairie Maloine, Paris-Montpellier, 1938, pp. 815-825.
13. Kaplan, M. M., and Meranze, D. R. Porcine virus encephalomyelitis and its possible biological relationship to human poliomyelitis. *Vet. Med.*, 1948, 43, 330-341.
14. Dobberstein, J. Histopathologie des Zentralnervensystems bei der Poliomyelitis des Schweines (ansteckende Schweinelähme, Teschener Krankheit). *Ztschr. f. Infektionskr.*, 1942, 59, 54-80.
15. Horstmann, D. M., Manuelidis, E., and Sprinz, H. Neuropathology of Teschen disease (virus encephalomyelitis of swine). *Proc. Soc. Exper. Biol. & Med.*, 1951, 77, 8-13.
16. Manuelidis, E. E., Horstmann, D. M., and Sprinz, H. Zur Histopathologie und Topik der experimentellen Teschener Krankheit. (Virus-Encephalomyelitis des Schweines). *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1952, 189, 208-230.
17. Horstmann, D. M. Experiments with Teschen disease (virus encephalomyelitis of swine). *J. Immunol.*, 1952, 69, 379-394.
18. Mussemeier, A. Die ansteckende Schweinelähme (Teschener Krankheit) und ihre Bekämpfung. *Berl. u. Münch. tierärztl. Wchnschr.*, 1940, 253-256.
19. Weil, A. *Textbook of Neuropathology*. Grune & Stratton, New York, 1945, ed. 2, p. 15.
20. Spielmeyer, W. Zur Histopathologie und Pathogenese der Poliomyelitis. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1932, 142, 159-199.
21. Scholz, W. Ueber herdförmige, protoplasmatische Gliawucherungen von syncytialem Charakter. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1922, 79, 114-179.
22. Környey, S., and Elek, P. Histologische Untersuchungen zur Pathogenese und Pathophysiologie der Teschener Krankheit. *Acta vet.*, 1952, 2, 143-161.
23. Seifried, O., and Spatz, H. Die Ausbreitung der encephalitischen Reaction bei der Bornaschen Krankheit der Pferde und deren Beziehungen zu der Encephalitis epidemica, der Heine-Medinschen Krankheit und der Lyssa des Menschen. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1930, 124, 317-382.
24. Spielmeyer, W. Infektion und Nervensystem. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1929-30, 123, 161-203.
25. Meyer, J. E. Über Befunde am Ganglion stellatum bei Kausalgie. *Klin. Wchnschr.*, 1947, 24-25, 372-374.
26. Bodian, D., and Howe, H. A. The significance of lesions in peripheral ganglia in chimpanzee and in human poliomyelitis. *J. Exper. Med.*, 1947, 85, 231-242.



27. Faber, H. K., Silverberg, R. J., and Dong, L. Studies on the entry and egress of poliomyelitic infection. I. Neurotropic infection of the peripheral ganglia in apparently healthy monkeys following casual exposure. *J. Exper. Med.*, 1950, 91, 417-424.
28. Spatz, H. Encephalitis. In: Bumke, O. *Handbuch der Geisteskrankheiten*. Julius Springer, Berlin, 1930, 11, 157-281.
29. Bodian, D. Histopathologic basis of clinical findings in poliomyelitis. *Am. J. Med.*, 1949, 6, 563-578.
30. Wolf, A. The Pathology of Some Viral Encephalitides. In: Kidd, J. G. (ed.). *The Pathogenesis and Pathology of Viral Diseases*. Columbia University Press, New York, 1950, pp. 194-213.
31. Haymaker, W., and Sabin, A. B. Topographic distribution of lesions in central nervous system in Japanese B encephalitis. Nature of the lesions, with report of a case on Okinawa. *Arch. Neurol. & Psychiat.*, 1947, 57, 673-692.
32. Zimmerman, H. M. The pathology of Japanese B encephalitis. *Am. J. Path.*, 1946, 22, 965-991.
33. Hurst, E. W. The transmission of "louping-ill" to the mouse and the monkey: histology of the experimental disease. *J. Comp. Path. & Therap.*, 1931, 44, 231-245.
34. Brownlee, A., and Wilson, D. R. Studies in the histopathology of louping-ill. *J. Comp. Path. & Therap.*, 1932, 45, 67-92.

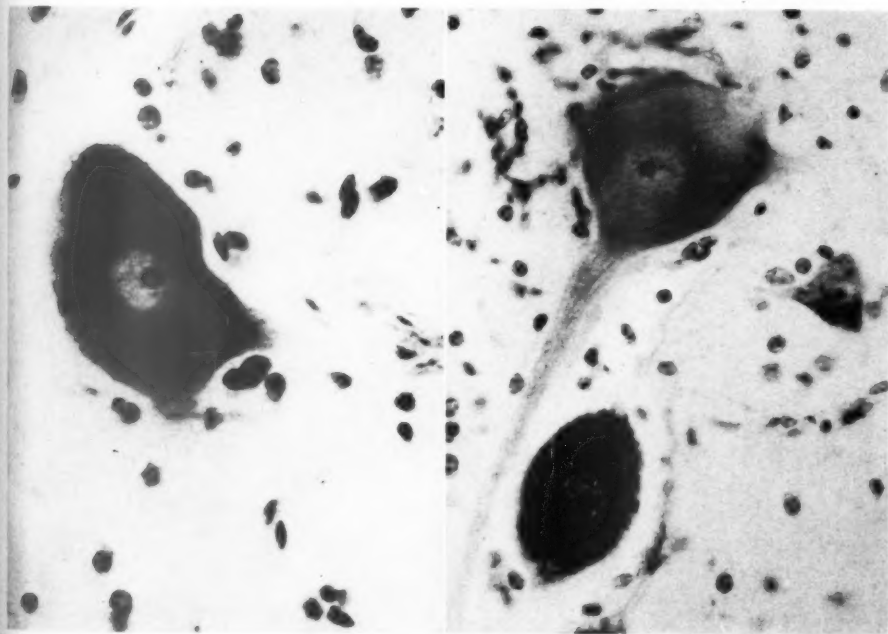
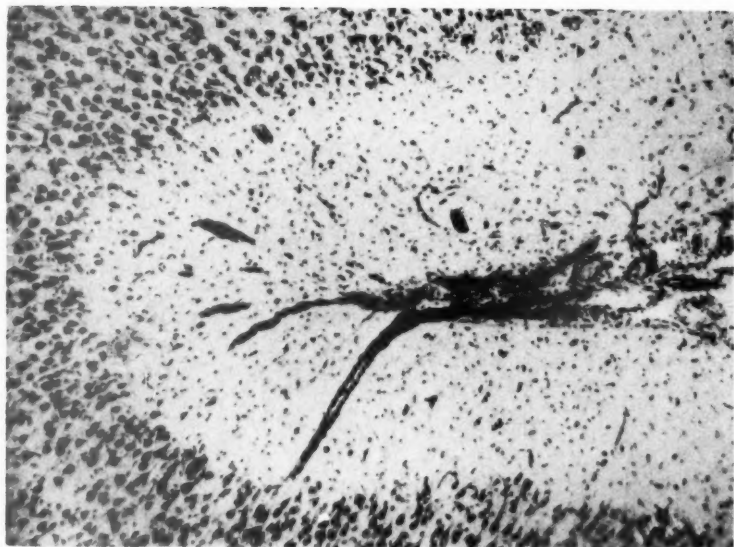
#### LEGENDS FOR FIGURES

- FIG. 1. Cerebral cortex. Mild meningitis and minimal perivascular infiltration in the first layer of the cortex.  $\times 70$ .
- FIG. 2. Anterior horn of spinal cord. The cell body shown exhibits a mild degree of neuronal degeneration. Nissl bodies hazy in outline. Slight hyperchromatism of nucleus.  $\times 600$ .
- FIG. 3. Anterior horn of spinal cord. In the perinuclear area and the cell periphery as well as in the nerve cell process, the Nissl substance is altered but still preserved. The hyperchromatism of the nucleus has progressed as compared with Figure 2. Partial chromatolysis is present.  $\times 600$ .





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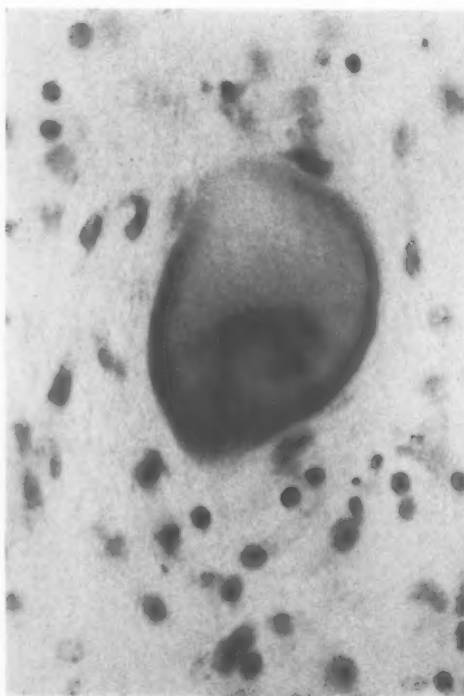
FIG. 4. Anterior horn of spinal cord. Almost complete chromatolysis with the nuclear membrane no longer visible.  $\times 600$ .

FIG. 5. Anterior horn of spinal cord. Central chromatolysis with peripheral vacuolization.  $\times 600$ .

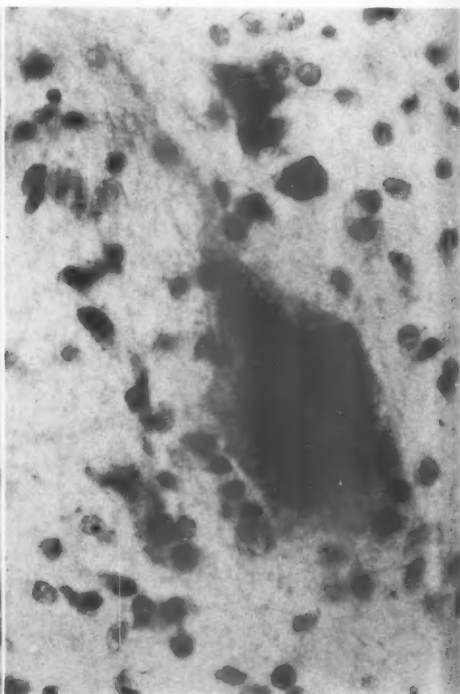
FIG. 6. Anterior horn of spinal cord. Complete vacuolization with dissolution of the cytoplasm and karyorrhexis.  $\times 530$ .

FIG. 7. Anterior horn of spinal cord. Hypertrophic, elongated, and twisted microglial cells.  $\times 940$ .

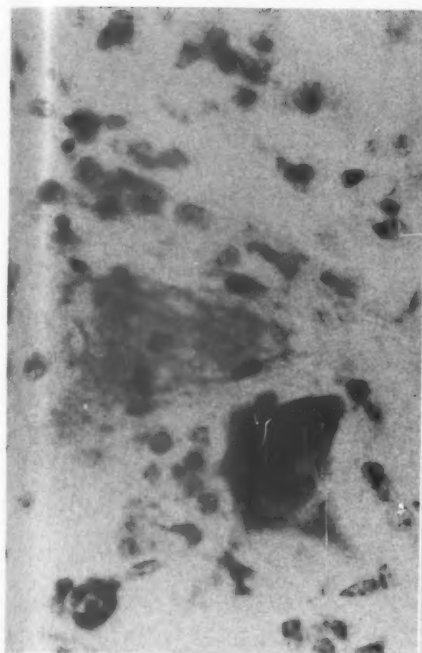
FIG. 8. Spinal cord. Many neuronophagic nodules and perivascular cuffing.  $\times 50$ .



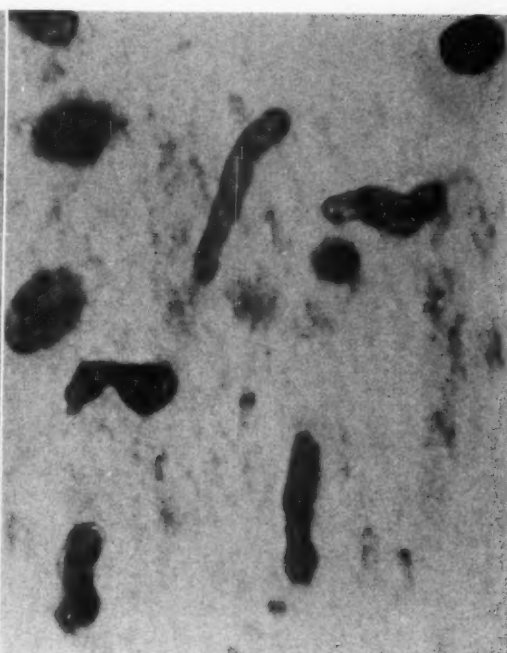
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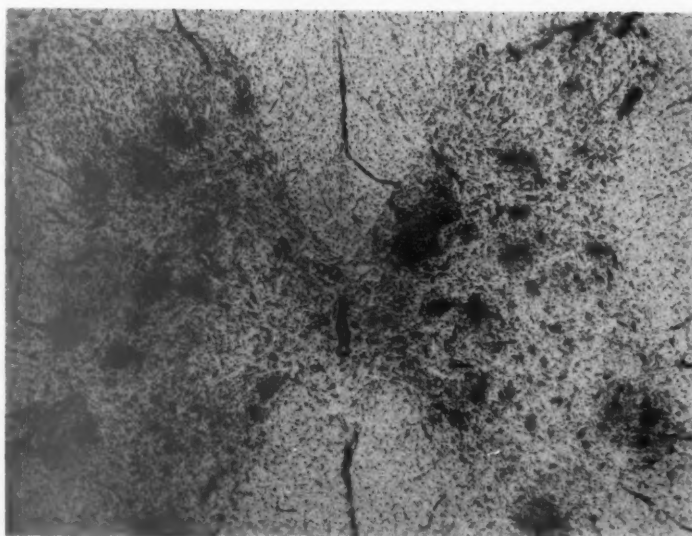
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FIG. 9. Anterior horn of spinal cord. Marked destruction of neurons. Many neuronophagic nodules. Intensive perivascular infiltration with lymphocytes.  $\times 90$ .

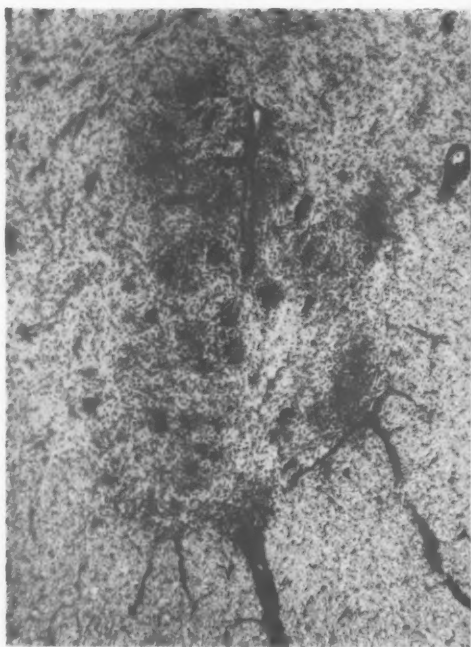
FIG. 10. Vacuolated Purkinje cells.  $\times 440$ .

FIG. 11. Cerebellum. (a) Complete destruction of the Purkinje cells with proliferation of the Bergmann glia. (b) Several Purkinje cells still remaining. In the molecular layer a few "Strauchwerke" are present.  $\times 85$ .

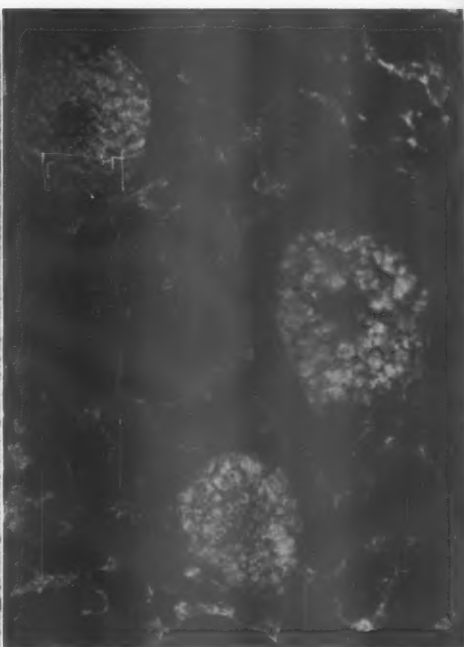
FIG. 12. Cerebellum. Numerous areas of neuronophagia and many cell nodules, especially in the Purkinje layer. Moderate perivascular cuffing.  $\times 42$ .

FIG. 13. Cerebral cortex. Perivascular infiltration and loose cell nodule. The nerve cells are very well preserved.  $\times 60$ .

FIG. 14. Cerebellar atrophy. Selective destruction of the layers of the cerebellar cortex with marked gliosis. A moderate meningitis is present.  $\times 42$ .



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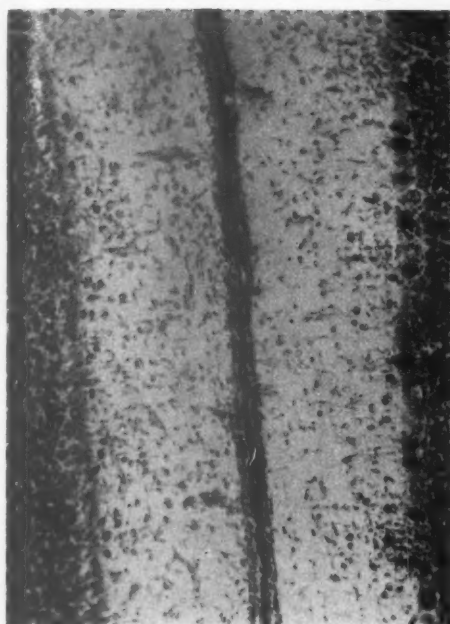


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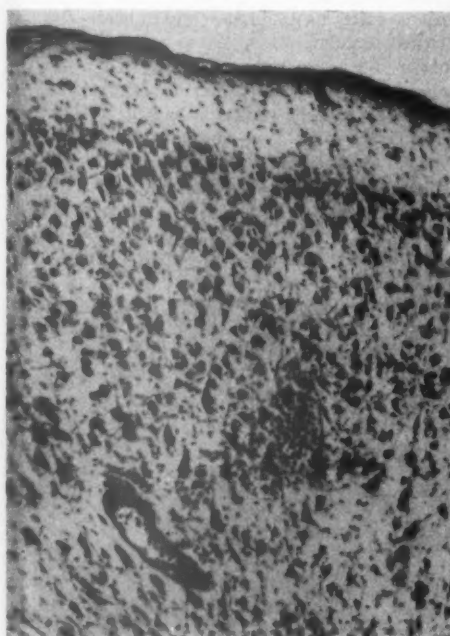
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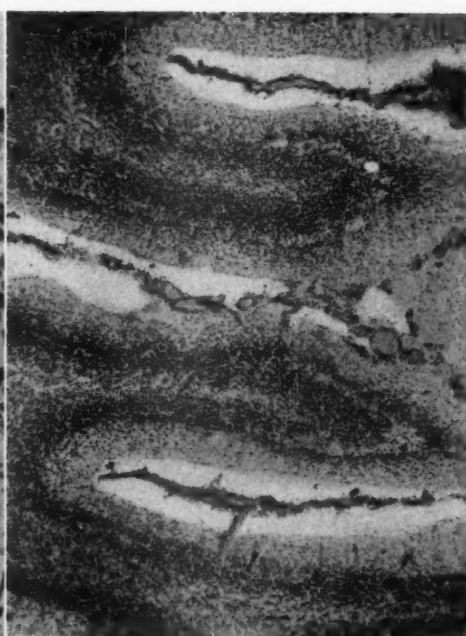
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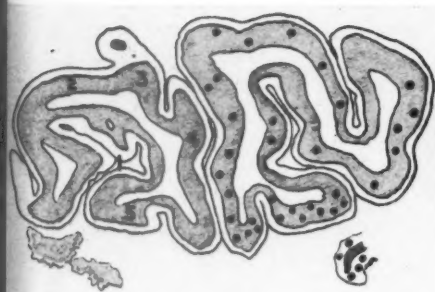
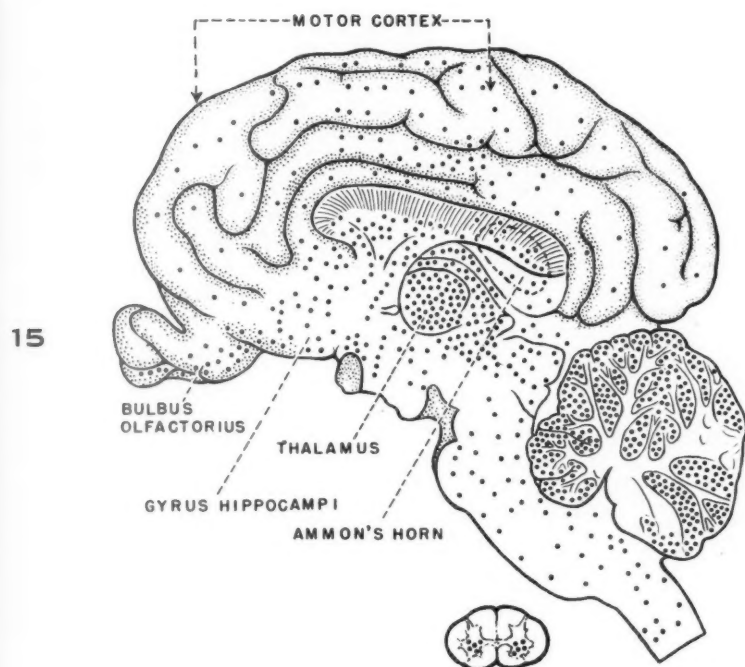
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FIG. 15. A composite diagram illustrating the distribution of the lesions (indicated by dots) in the pig brain and medulla.

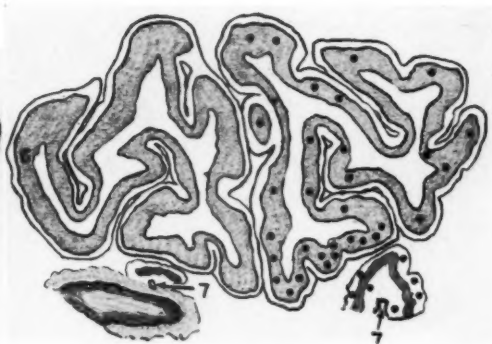
FIGS. 16 to 38. Figures 16 to 35 represent drawings of transverse sections of the pig brain, Figure 16 being most anterior (frontal lobe) and Figure 35 most posterior (medulla). Figs. 28 and 29. Transverse sections of the occipital lobe. Figs. 30 to 34. Transverse sections of the cerebellum. Fig. 36. Transverse section of the cervical cord. Fig. 37. Transverse section of the thoracic cord. Fig. 38. Transverse section of the lumbar cord. The numbers (1 to 87) on the left side of the drawings indicate the following anatomical regions of the pig brain, while the dots indicate the distribution of lesions: 1. sulcus praesylyvius; 2. gyrus sigmoideus posterior; 3. gyrus lateralis; 4. gyrus genualis; 5. gyrus proreus; 6. gyrus coronalis; 7. cavity of the olfactory bulb; 9. attachment of the bulb to the base of the brain; 10. gyrus hippocampi; 11. lateral ventricle; 12. motor cortex; 13. claustrum; 14. nucleus caudatus; 15. undifferentiated matrix cells around the lateral ventricle; 16. putamen; 17. capsula externa; 18. capsula interna; 19. nuclei septi pellucidi; 20. optic nerve; 21. corpus callosum; 22. gyrus rostralis; 23. gyrus fornicatus; 24. gyrus lateralis; 25. sulcus lateralis; 26. gyrus suprasylvius; 27. gyrus suprasylvius anterior; 28. gyrus obitalis; 29. sulcus suprasylvius; 30. third ventricle; 31. chiasma opticum; 32. optic tract; 33. fornix; 34. thalamus; 35. pallidum; 36. nucleus amygdalae; 37. temporal horn of the lateral ventricle; 38. Ammon's horn; 39. fascia dentata; 40. corpora geniculata; 41. corpus quadrigeminum anticum; 42. substantia grisea centralis; 43. aqueductus Sylvii; 44. Westfall-Edinger nucleus; 45. nucleus nervi oculo-motorii; 46. substantia nigra; 47. nucleus ruber; 48. pes pedunculi cerebri; 49. corpus pineale; 50. formatio reticularis; 51. nucleus ventralis thalami; 52. fourth ventricle; 53. posterior horn of the lateral ventricle; 54. vermis; 55. cerebellar hemisphere; 56. corpus quadrigeminum posticum; 57. nuclei pontis lateralis, medialis and ventralis; 58. nucleus Gudden; 59. ganglion interpedunculare; 60. nucleus dorsalis raphe tegmenti; 61. nucleus ventralis and dorsalis brachii conjunctivi; 62. nucleus nervi trochleari; 63. nucleus proprius substantiae griseae centralis; 64. nuclei dorsalis, medialis and ventralis lemnisci lateralis; 65. lingula cerebelli; 66. nucleus motorius and sensibilis nervi trigemini; 67. nucleus Bechterew; 68. brachium pontis; 69. flocculus cerebelli; 70. radix mesencephalica nervi trigemini; 71. nucleus ventralis raphe tegmenti; 72. nucleus ventralis formatio reticularis; 73. formatio reticularis lateralis; 74. nucleus lateralis formatio reticularis; 75. nuclei trapezoidi; 76. nucleus paraolivaris; 77. nucleus olivaris inferior; 78. nucleus funiculi lateralis; 79. substantia gelatinosa; 80. nucleus ambiguus inferior; 81. nucleus nervi VII; 82. nuclei nervi XI; 83. cellulae zonales; 84. cellulae terminales; 85. cellulae Gierke; 86. nucleus Goll; 87. nucleus Burdach.



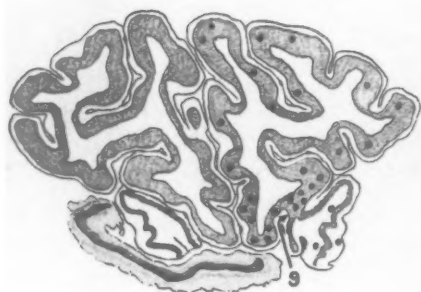




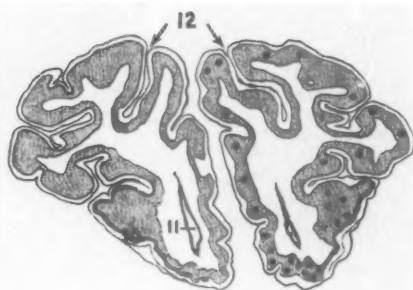
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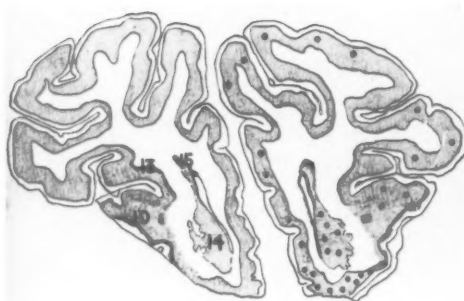
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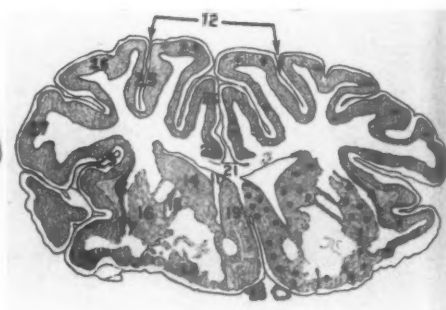
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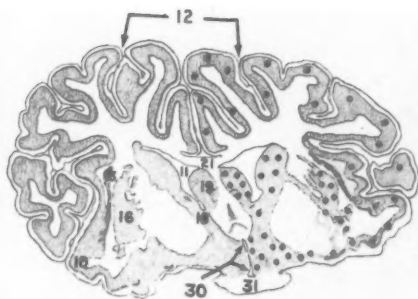
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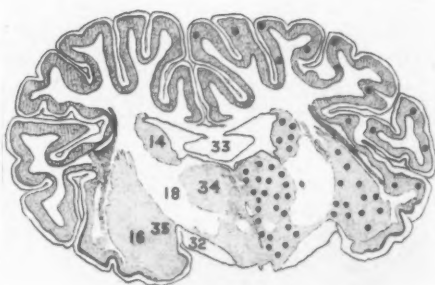
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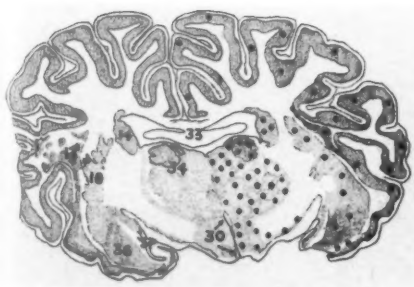
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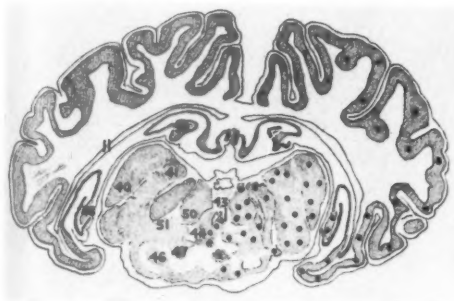
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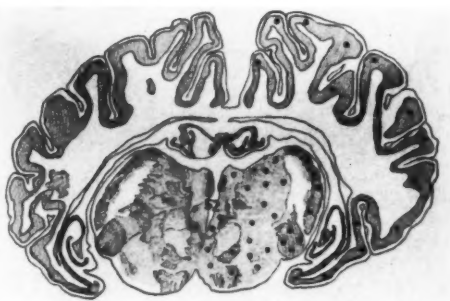
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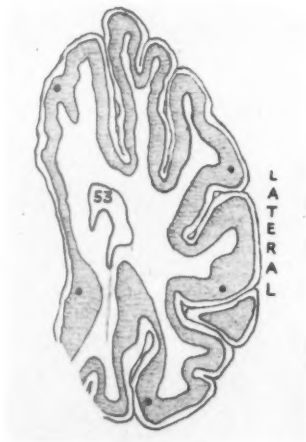
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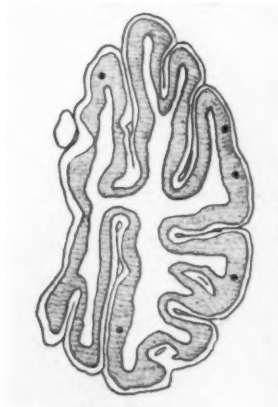
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FIGS. 30 to 38. The numbers refer to anatomical regions as listed on page 592; the dots designate the distribution of lesions as identified.

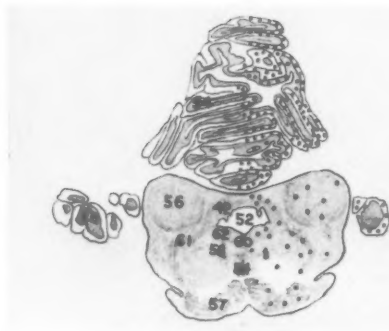
FIGS. 30 to 34. Transverse sections of the cerebellum.

FIG. 35. Most posterior transverse section of brain (medulla).

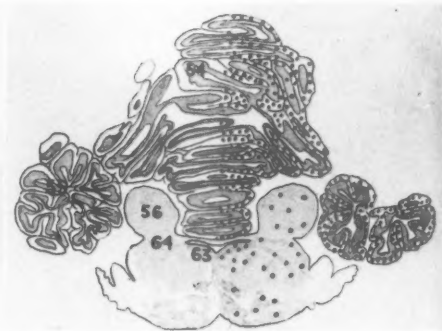
FIG. 36. Transverse section of the cervical cord.

FIG. 37. Transverse section of the thoracic cord.

FIG. 38. Transverse section of the lumbar cord.



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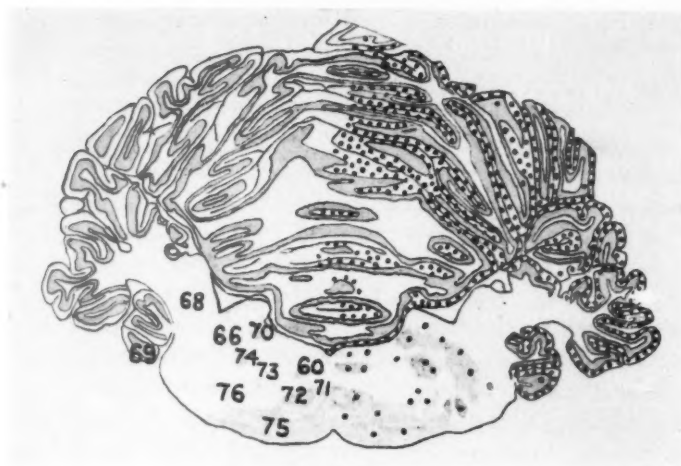
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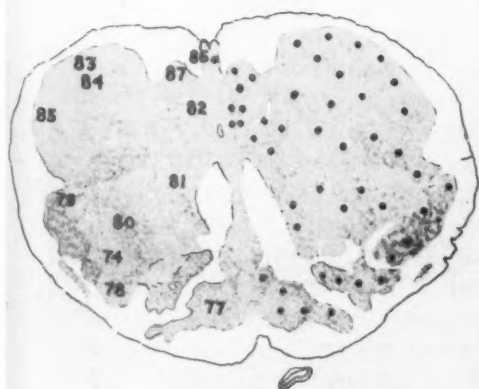
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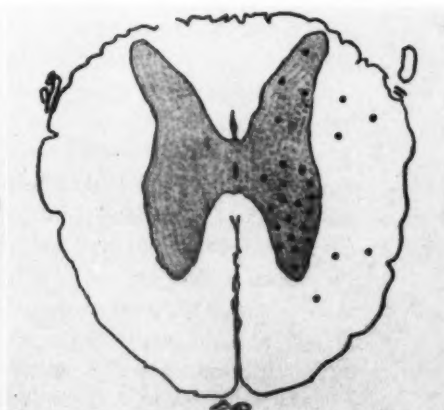
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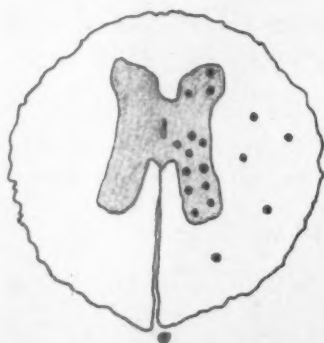
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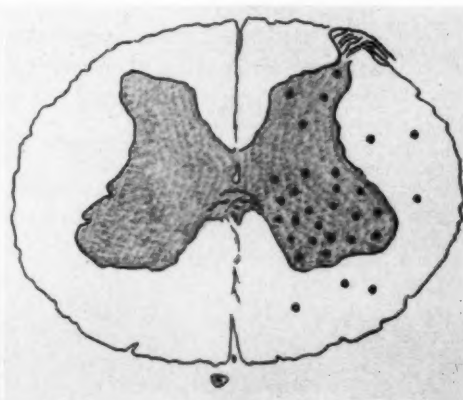
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## THYROID CHANGES IN ACUTE EXPERIMENTAL CHAGAS' DISEASE IN DOGS \*

FRANS C. GOBLE, Sc.D.

(From the Research Division, Abbott Laboratories, North Chicago, Ill.)

The views of the pathogenesis of American trypanosomiasis set forth by Chagas<sup>1,2</sup> became the subject of considerable controversy during the decade following the publication of the account of his observations in Minas Geraes. The attitude of Kraus<sup>3</sup> may be said to summarize the scepticism which developed concerning the relation of *Trypanosoma cruzi* to the etiology of goiter, idiocy, aphasia, paralysis, cerebral diplegia, myxedema, and infantilism which Chagas included in the symptomatology of the disease which now bears his name. Further studies, reviewed by Yorke,<sup>4</sup> led to the current tendency to consider many of the clinical phenomena originally associated with chronic Chagas' disease to be the result of the coincidental occurrence of *T. cruzi* infection in areas of endemic goiter.

Romaña<sup>5</sup> pointed out that none of the many investigators of the experimental infection in animals had demonstrated that the parasite had any predilection for the thyroid gland nor had they observed hypertrophy in that organ as a consequence of the disease. Mazza and Jörg<sup>6</sup> reported "myxoedema" in a naturally infected dog, but Collier, Fulton, and Innes<sup>7</sup> have attributed the edematous manifestations of the disease to "the common inflammatory oedema which occurs not only in trypanosomiasis but in many other protozoal, bacterial, and virus conditions." These authors reported that the thyroid glands of infected mice showed neither parasitic invasion nor tissue change.

During the course of recent studies on experimental Chagas' disease in dogs,<sup>8</sup> nearly complete necropsies were performed on most of the animals infected. Microscopic examination of the tissues from these dogs revealed, in addition to the pathologic processes in the viscera, brain, and reticulo-endothelial system which have been described by other authors, abnormalities in the thyroid structure of several of the animals. In view of the conflicting opinions which have been cited on the relation of *T. cruzi* to thyroid dysfunction, these findings are considered to be of interest and form the basis for the present report.

### MATERIALS AND METHODS

The "Brazil" strain of *T. cruzi* Chagas, 1909, was used for the infection of all the animals considered here. The parasite was grown in

\* Received for publication, September 16, 1953.

a diphasic medium, previously described,<sup>9</sup> and 2-week old overlay, containing 100,000,000 organisms per ml., was used as inoculum. Dogs were infected by injection (subcutaneous, except as will be noted) of 100,000,000 culture forms per kg. of body weight.

All of the dogs used were mongrels, but attempts were made to minimize other than genetic variables, and to standardize the handling of the animals. The juveniles used were all born and raised in the laboratory and were infected at the age of 10 weeks. The adults were all short haired, of apparent hound-terrier ancestry, selected for uniformity in size. All animals were dewormed (with tetrachloroethylene) and given distemper prophylaxis (Green distemperoid virus) at least 2 weeks prior to infection.

The blood of each infected animal was examined daily throughout the pre-patent period, and during the patent period daily trypanosome counts were made on wet preparations. Animals which died or were sacrificed were necropsied and tissues were fixed in 10 per cent formalin. Paraffin sections were stained with hematoxylin and eosin. The organs ordinarily examined were heart, lungs, liver, spleen, kidneys, pancreas, stomach, intestine, lymph nodes, thyroid and adrenal glands, pituitary body, brain, spinal cord, and gonads.

Tissues from the following dogs were studied: 34 juveniles (17 males, 17 females) infected subcutaneously at 10 weeks of age; 6 juveniles (females) uninfected, necropsied between 3 and 13½ weeks of age; 7 adults (3 males, 4 females) infected subcutaneously; 2 adults (females) infected intraperitoneally; 2 adults (1 male and 1 female) infected intravenously. Tissues of many normal control dogs, uninfected and non-medicated, were available from previous studies conducted in connection with subacute and chronic toxicity tests on new therapeutic agents.

Eighteen of the young dogs (9 males, 9 females) received no treatment whatsoever. The rest of the juveniles received compounds, at dose levels known from previous studies to be non-toxic, which did not affect the course of the disease. With one exception these were all preparations which have not, in our experience, proved satisfactory for the treatment of experimental Chagas' disease in dogs. The accepted product was pentaquine, which has been effectively used in treating *T. cruzi* infections in dogs.<sup>8</sup>

#### OBSERVATIONS

Juvenile dogs, untreated or given non-toxic but ineffective medication, died from acute myocarditis and cardiac insufficiency between 25 and 49 days after infection (animals were 13½ to 17 weeks old at death).

Thyroid glands from animals (17 males, 17 females) which died during this period showed, upon histologic examination, striking differences dependent on sex. Those from all of the males but one were normal as compared with material from uninfected, non-medicated controls. Those from females, however, showed diffuse parenchymatous changes in 12 of the 17 examined.

The times of death and status of the thyroid glands are shown in Table I along with the periods of patency and the compounds used in

TABLE I  
*Patency, Survival Time, and Incidence of Thyroid Abnormalities in Juvenile Dogs Which Succumbed to Experimental Chagas' Disease*

Males				Females			
Survival time	Patent period	Treatment*	Thyroid abnormality	Survival time	Patent period	Treatment*	Thyroid abnormality
days	days			days	days		
26	13	None	—	25	6	None	+
27	14	None	—	28	7	None	—
29	10	None	+	29	14	None	+
30	9	None	—	30	8	None	+
31	6	None	—	32	13	None	+
32	12	None	—	32	17	None	+
34	19	None	—	37	13	None	+
38	13	None	—	44	24	None	+
41	25	None	—	49	39	None	—
26	9	Pentaquine	—	26	9	Pentaquine	—
26	10	Potassium iodide	—	26	6	Isopentaquine	+
27	8	Isopentaquine	—	27	9	Potassium iodide	—
28	8	Potassium iodide	—	28	7	Primaquine	+
29	16	Potassium iodide	—	29	9	Isopentaquine	+
30	9	Bayer 7602	—	33	15	Pentaquine	—
30	10	Bayer 7602	—	34	13	Potassium iodide	+
33	10	Compound Q	—	43	30	Primaquine	+

\* The synthetic organic compounds used in the above treatments were:

- Pentaquine, 6-methoxy-8-(5-isopropylaminoamylamino)-quinoline, (SN-13276).
- Isopentaquine, 6-methoxy-8-(1-methyl-4-isopropylaminobutylamino)-quinoline, (SN-13274).
- Primaquine, 6-methoxy-8-(1-methyl-4-aminobutylamino)-quinoline, (SN-13272).
- Bayer 7602, bis-(2-methyl-4-amino-6-quinolyl)-diallylmalonamide.
- Compound Q, a 6-methoxy-8-aminoquinoline related to SN-5838.

treatment. The dogs have been grouped according to sex and treatment in Table I in order to indicate more clearly the histories of the animals from which material was examined and to show that the groups were roughly comparable. These data on the survival times, patent periods, and treatment, plus additional information on degree of parasitemia

and extent of lesions in the organs, indicated that the presence or absence of thyroid abnormality was more closely correlated with sex than with any other factor.

On gross examination the affected thyroid glands usually were normal in size, configuration, and consistency. Microscopically, however, the follicles were small and contained little or no colloid (Fig. 2). The epithelium was uniformly cuboidal and showed no tendency to become columnar or to form proliferative papillae. Many of the follicles contained spherical agranulocytic cells with eccentric nuclei (Figs. 3 and 4). There was no evidence of acute inflammatory process, fibrosis of either capsule or stroma, or invasion of the organ by *T. cruzi* organisms. The changes were diffuse rather than focal.

Only one young male showed this thyroid abnormality. Five of the 17 females did not have the condition. Two of these were non-medicated controls, one had received 100 mg. per kg. per day of potassium iodide from the first day of parasitemia, and 2 had been treated with pentaquine in doses too low to effect cure. The lack of tissue change in the thyroid gland of the animal which received potassium iodide is not considered to be attributable to the medication inasmuch as another female on the same regimen suffered thyroid damage. It does seem possible, however, that the pentaquine treatment, even though inadequate, modified the effect of the disease on the thyroid gland.

In addition to the juveniles indicated in Figure 1, 6 uninfected females less than 13½ weeks old were examined as a control on possible histologic differences attributable to age. The thyroid glands of these dogs showed no deviations from the conditions recognized as normal (Fig. 1) in material from uninfected adults and in the infected juvenile males. Likewise, no abnormalities were observed in the thyroid glands of 6 juvenile females which survived the acute myocarditic stage of the disease by virtue of adequate treatment with pentaquine and were necropsied at various times between 54 and 95 days after infection.

Seven adult dogs were infected subcutaneously, 3 males and 4 females. All of the males (untreated) succumbed between 35 and 38 days after infection and in one of these thyroid changes similar to those which have been described were noted. Three of the females were untreated but only one died (at 32 days). This animal showed no thyroid abnormality. The other two and a medicated female lived to 95 days, at which time they were sacrificed. Their tissues were normal. Two adult females were infected intraperitoneally, and one of them was treated with pentaquine. The untreated dog died at 37 days and



showed the thyroid changes; the other was necropsied at 68 days and the thyroid gland was found to be normal. Two adults, a female and a male, were infected intravenously. Their thyroid glands were normal at necropsy at 30 and 40 days, respectively, after infection. Although the number of adults examined was small, there seemed to be no correlation between the degree of parasitemia or length of the patent period and the presence of thyroid abnormality.

#### DISCUSSION

The changes observed in dogs with experimental Chagas' disease resemble most closely those reported by Cole and Womack<sup>10</sup> in the thyroid glands of dogs with various infections and toxemias. These were illustrated in their later publications.<sup>11-13</sup> The cells which appear to lie free in the follicles, however, do not seem to be the "desquamated" epithelial cells described by Cole *et al.*, but more closely resemble the macrophages which sometimes occur in the acini in nodular goiter<sup>14</sup> or in colloidophagy stimulated by excess of thyrotropic hormone.<sup>15</sup>

Cole *et al.*<sup>10-13</sup> did not indicate that sex was a factor to be considered in their experimental work, but McCarrison<sup>16</sup> noted that female rats receiving goitrogenic diets were more susceptible to thyroid changes than males. It is, of course, common knowledge that all forms of goiter are more commonly encountered in women than in men. The hypersusceptibility to thyroid changes of female dogs with Chagas' disease is noteworthy both in its prepuberal occurrence and because of the fact that females are generally more refractory than males to experimental infections with *T. cruzi*. In adult dogs<sup>8</sup> and in mice<sup>17,9</sup> this resistance is manifested by slightly longer pre-patent periods, lower parasitemias, and longer survival times in females. Similar observations would be difficult to make on spontaneous cases in humans, but clinical investigators have not reported that either the incidence of the disease or its course is affected by the sex of the patient. The facts that differences in the host-parasite relationship dependent on sex occur prepuberally in both dogs and mice<sup>9</sup> and that the administration of testosterone to female mice and progesterone, estrone, or diethylstilbestrol to male mice does not alter the course of the disease<sup>18</sup> seem to indicate that substances other than the steroids of the adult gonads are concerned.

Martins<sup>19</sup> found that when patients with Chagas' disease were treated by intravenous administration of sodium iodide the intensity of their serologic reaction (Machado-Guerreiro test) was reduced. In

one case it became negative. He did not attempt to explain the mechanism nor did he imply that cure was effected. The attempts at treatment with potassium iodide in the present study were suggested in part by Martins' report and in part by the observations of Cole and Womack<sup>12</sup> who found that the oral administration of Lugol's solution to bacterially infected dogs tended to protect against the thyroid "hyperplasia, desquamation and loss of colloid which so constantly accompanies severe infections."

It is difficult to compare thyroid changes in the dog with those in man because of certain differences in structure and response. The amount of connective tissue in the thyroid gland of the dog is usually considerably less than that found in human glands<sup>13</sup> and the changes observed in humans "who died from infections . . . are not nearly as constant as those noted in animals."<sup>11</sup> The experimental production of thyroid abnormalities of a type which may precede the development of goitrous conditions, reported by the authors mentioned,<sup>10-13</sup> and the demonstration of similar changes in experimental Chagas' disease, lead to resumed speculation of the possible relation of *T. cruzi* infections to the thyroid dysfunctions<sup>20</sup> observed in the areas where American trypanosomiasis occurs.

The lack of invasion or inflammation of the organ by the parasite is not inconsistent with the theory of Rocha Lima and P. Chagas that the trypanosome produces toxins which are concerned in pathogenesis.<sup>21</sup> The development of pregoitrous lesions by toxemias of bacterial origin or by injection of simple substances such as histamine and glycine<sup>11</sup> or purine bases and their derivatives<sup>22</sup> is likewise brought about without direct local physical or microbial action on the thyroid cells. Inasmuch as no correlation could be found during the present study between the severity of the infection as manifested by the survival times, parasitemia, or extent of lesions in other organs, and the thyroid status of the animals which succumbed, the sites of origin of possible thyrotoxic substances are not apparent.

#### REFERENCES

1. Chagas, C. Nova entidade morbida do homem. Resumo geral de estudos etiológicos e clínicos. *Mem. Inst. Oswaldo Cruz*, 1911, 3, Pt. 2, 219-275.
2. Chagas, C. Processos patojenicos da tripanozomíase americana. *Mem. Inst. Oswaldo Cruz*, 1916, 8, Pt. 2, 5-36.
3. Kraus, R. Die Chagaskrankheit, Kropf und Kretinismus in Südamerika. *Wien. klin. Wchnschr.*, 1926, 39, 378-382.
4. Yorke, W. Chagas' disease. A critical review. *Trop. Dis. Bull.*, 1937, 34, 275-300.

5. Romañá, C. Tripanosomiasis americana y bocio endémico. Estado actual de la cuestión. *Semana méd.*, 1935, 42, 897-902.
6. Mazza, S., and Jörg, M. E. Infección natural mortal por *S. cruzi* en cachorro de perro "Pila" de Jujuy. En Homenaje a la Memoria de Carlos Chagas, Novena Reunión de la Sociedad Argentina de patología Regional, Mendoza, 1936, 1, 365-411.
7. Collier, H. O. J., Fulton, J. D., and Innes, J. R. M. The oedema of mice infected with *Trypanosoma cruzi*, and the accompanying pathological lesions. *Ann. Trop. Med.*, 1942, 36, 137-150.
8. Goble, F. C. Observations on experimental Chagas' disease in dogs. *Am. J. Trop. Med.*, 1952, 1, 189-204.
9. Goble, F. C. Studies on experimental Chagas' disease in mice in relation to chemotherapeutic testing. *J. Parasitol.*, 1951, 37, 408-414.
10. Cole, W. H., and Womack, N. A. The thyroid in infections and toxemias. *Proc. Soc. Exper. Biol. & Med.*, 1927-28, 25, 188-191.
11. Cole, W. H., Womack, N. A., and Gray, S. H. The thyroid in infections and toxemias. Pathological changes in the human gland. *Am. J. Surg.*, 1929, 6, 221-229.
12. Cole, W. H., and Womack, N. A. Reaction of the thyroid gland to infections in other parts of body. *J. A. M. A.*, 1929, 92, 453-457.
13. Womack, N. A., and Cole, W. H. Normal and pathologic repair in the thyroid gland. *Arch. Surg.*, 1931, 23, 466-476.
14. Boyd, W. Surgical Pathology. W. B. Saunders Co., Philadelphia, 1942, ed. 5, p. 189.
15. Hellwig, C. A. Colloidophagy in the human thyroid gland. *Science*, 1951, 113, 725-726.
16. McCarrison, R. The experimental production of a new type of goitre unrelated in its origin to iodine. *Lancet*, 1927, 1, 916-920.
17. Hauschka, T. S. Sex of host as a factor in Chagas' disease. *J. Parasitol.*, 1947, 33, 399-404.
18. Goble, F. C. Lack of effect of sex hormones on the course of experimental Chagas' disease in mice. *J. Parasitol.*, 1952, 38 (suppl. to no. 4, sect. 2), 15.
19. Martins, F. Modificações verificadas em serodiagnósticos da doença de Chagas com o iodeto de sódio. *Med. cir. farm.*, 1949, no. 163, 656-659.
20. Azevedo, A. P. de. Histologia pathologica da glandula thyreoide na forma aguda da molestia de Chagas (Trypanosomose americana). *Mem. Inst. Oswaldo Cruz*, 1933, 27, 93-123.
21. Crowell, B. C. The acute form of American trypanosomiasis: notes on its pathology, with autopsy report and observations on trypanosomiasis cruzi in animals. *Am. J. Trop. Med.*, 1923, 3, 425-454.
22. Cole, W. H., Womack, N. A., and Ellett, W. H. The production of hyperplasia of the thyroid gland by chemical means with special reference to purine bases and their derivatives. *Arch. Surg.*, 1931, 22, 926-935.

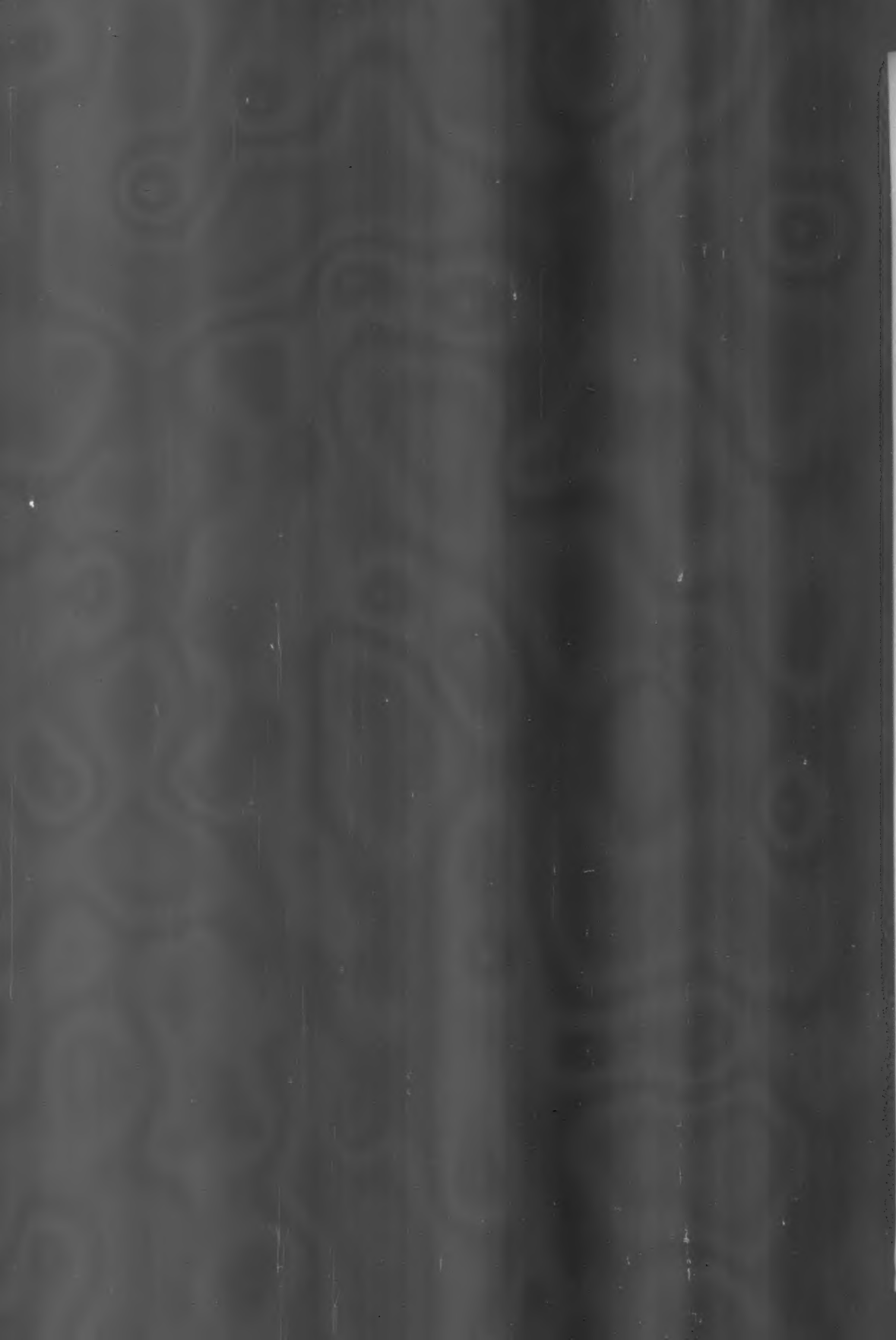
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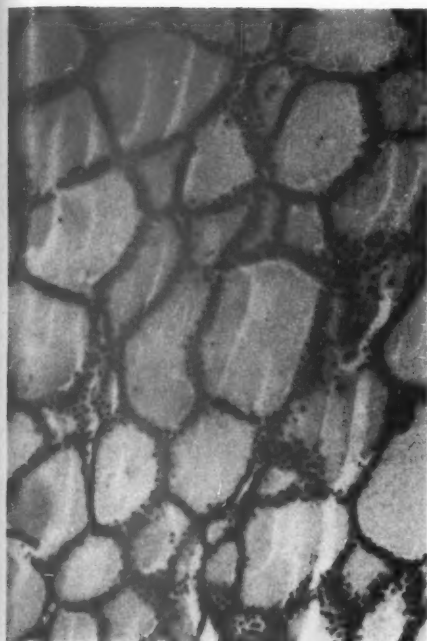
[ Illustrations follow ]

## LEGENDS FOR FIGURES

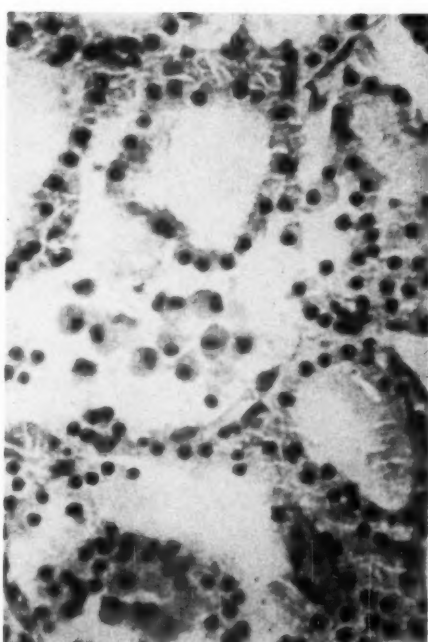
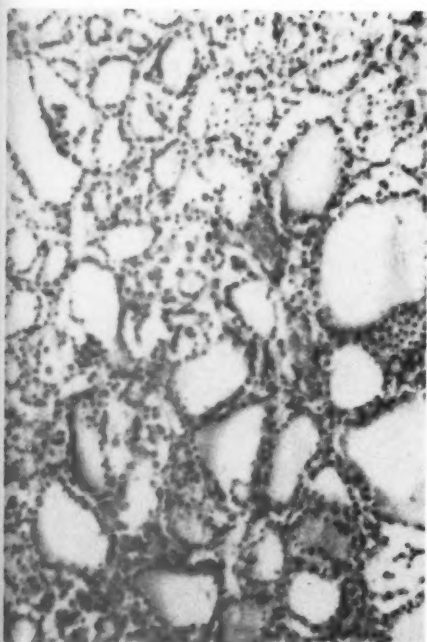
- FIG. 1. Normal thyroid gland, from a female dog (13½ weeks old).  $\times 120$ .
- FIG. 2. Thyroid gland from a female dog (13½ weeks old) which died 25 days after infection with *Trypanosoma cruzi*.  $\times 120$ .
- FIG. 3. Thyroid gland from a female dog (14½ weeks old) which died 32 days after infection with *T. cruzi*.  $\times 120$ .
- FIG. 4. High-power view of the thyroid gland used for Figure 3, showing colloidophagous cells in the lumina of the follicles.  $\times 905$ .





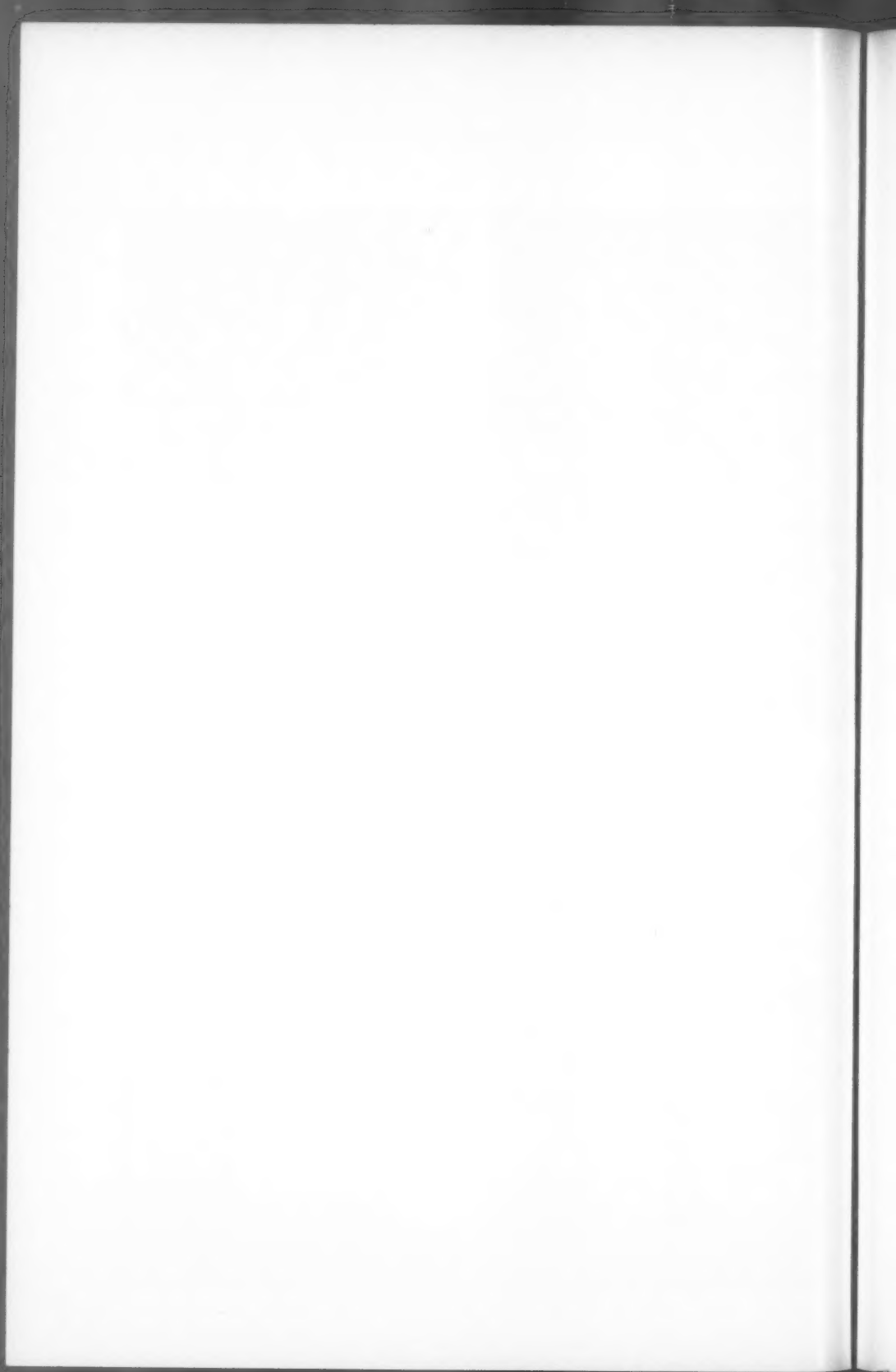


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FIFTY-FIRST ANNUAL MEETING  
OF THE  
AMERICAN ASSOCIATION OF PATHOLOGISTS  
AND BACTERIOLOGISTS  
PHILADELPHIA  
APRIL 8TH, 9TH, AND 10TH, 1954



THE AMERICAN ASSOCIATION OF PATHOLOGISTS  
AND BACTERIOLOGISTS

Fifty-first Annual Meeting,  
Benjamin Franklin Hotel,  
Philadelphia

April 8th, 9th, and 10th, 1954

PRESIDENT McNAUGHT IN THE CHAIR

BUSINESS MEETING

April 8, 1954

The following nominations for elective officers were submitted by the Council:

<i>President</i>	DR. G. LYMAN DUFF
<i>Vice-President</i>	DR. EDWIN W. SCHULTZ
<i>*Secretary</i>	DR. EDWARD A. GALL
<i>Treasurer</i>	BRIG. GEN. ELBERT DECOURSEY
<i>Incoming Member of Council</i>	DR. ALAN R. MORITZ

Additional nominations were called for. None having been offered, it was moved and seconded from the floor that the Secretary be instructed to cast a unanimous ballot for the entire slate.

At the direction of the President, the Secretary reported the following actions of the Council:

Election of New Members

Stanley M. Aronson, New York, N.Y.	Willard H. Eyestone, Chevy Chase, Md.
Ronald S. Beckett, Hartford, Conn.	Emmanuel Farber, New Orleans, La.
Morgan Berthrong, Colorado Springs, Colo.	Edwin R. Fisher, Cleveland, Ohio
Virgil R. Bleisch, Trenton, Ill.	Fred P. Handler, University City, Mo.
William N. Campbell, Elkins Park, Pa.	Adolf Hochwald, New York, N.Y.
Fremont E. Davis, Los Angeles, Calif.	Dwight M. Kuhns, Silver Spring, Md.

\* Effective January 1, 1955.

Marvin Kuschner, New York, N.Y.	Elmer E. Pautler, Jr., Memphis, Tenn.
Harrison Latta, Cleveland, Ohio	Leandro Potenza, Caracas, Venezuela
Raymond J. Leffler, New York, N.Y.	Walter G. Rice, St. Louis, Mo.
Seymour Levine, Cincinnati, Ohio	Goetz W. Richter, New York, N.Y.
Paul H. Lober, Minneapolis, Minn.	Oscar A. Ross, Cleveland, Ohio
William V. Lovitt, Jr., Baltimore, Md.	Albert G. Smith, Durham, N.C.
Leo Lowbeer, Tulsa, Okla.	Vernie A. Stenbridge, Galveston, Texas
Ross C. MacCardle, Bethesda, Md.	Stephen S. Sternberg, Brooklyn, N.Y.
Lawrence J. McCormack, Cleve- land, Ohio	Louis B. Thomas, Bethesda, Md.
George E. Murphy, New York, N.Y.	Theodore Winship, Washington, D.C.
Eli M. Nadel, Bethesda, Md.	John P. Wyatt, Kirkwood, Mo.
William B. Ober, Washington, D.C.	Joseph M. Young, Memphis, Tenn.
M. David Orrahood, Glendale, Mo.	Frederick G. Zak, Manhasset, N.Y.

With deep regret, the recording of the deaths of William S. Quinland, William L. Robinson, Albert E. Steele, and S. Burt Wolbach.

The re-election of Dr. Carl V. Weller as Editor-in-Chief of *The American Journal of Pathology* for a period of seven years, effective January 1, 1955.

The election of Dr. Alvin J. Cox, Jr., to the Editorial Board of *The American Journal of Pathology* for a period of six years, beginning January 1, 1955.

The re-election of Miss Dorothy E. Seiferlein as Editorial Assistant of *The American Journal of Pathology* for the ensuing year.

The nomination of Dr. Granville A. Bennett as representative of the Association in the Division of Medical Sciences of the National Research Council for a period of three years, effective July 1, 1954.

The nomination of Dr. Jerome T. Syverton as representative of the Association on the American Type Culture Collection of the American Association of Parasitologists and Bacteriologists.

The designation of Dr. Jerome T. Syverton and Dr. Thomas M. Rivers as delegates of the Association to the International Poliomyelitis Congress to be held in Rome on September 6 to 10, 1954.

The Secretary announced that the next annual meeting of the Asso-

ciation will be held in Houston, Texas, on April 7, 8, and 9, 1955. The topic for the symposium is "New Approaches to the Study of Renal Diseases."

The Secretary further announced that the annual meeting in 1956 would be held in Cincinnati, Ohio; the dates to be announced later.

The President then asked if there were any business from the floor. None was presented, and the business meeting adjourned.

Alan R. Moritz, *Secretary*

#### REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted. It was accompanied by a letter of certification from Frank D. Flynn, Auditor, Melrose, Massachusetts. In condensed form, the Treasurer's report follows:

##### General Checking Account

###### *Receipts*

Balance on hand, January 1, 1953 .....	\$ 5,130.40
Membership dues .....	\$ 8,353.75
Interest on bonds .....	500.00
	<hr/>
	8,853.75
	<hr/>
	\$13,984.15

###### *Disbursements*

American Journal of Pathology .....	\$ 7,160.00
Secretary's office, clerical .....	\$300.00
Printing, travel, supplies .....	526.55
Abstracts, clerical .....	65.00
	<hr/>
	891.55
Treasurer's office, clerical .....	\$150.00
Supplies .....	76.50
Auditing and deposit box .....	42.20
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	268.70
	<hr/>
	8,320.25

Balance on hand, December 31, 1953 .....	\$ 5,663.90
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##### Investment Account

Balance, January 1, 1953 .....	\$34,325.83
Interest on savings accounts .....	520.50
	<hr/>
Balance, December 31, 1953 .....	\$34,846.33

##### Inventory

U.S. bonds, series G .....	\$20,000.00
Providence Institute for Savings .....	4,272.11
Franklin Savings Bank .....	4,214.17
Cambridge Savings Bank .....	4,319.75
National Shawmut Bank .....	2,040.30
	<hr/>
	\$34,846.33

Sidney Farber, *Treasurer*





## SCIENTIFIC PROCEEDINGS

**THE LOCAL ACTION OF GROWTH HORMONE UPON GRANULATION TISSUE FORMATION.** R. B. Stebbins (by invitation) and H. C. Stoerk, Merck Institute for Therapeutic Research, Rahway, N.J.

*Abstract.* By making use of the simple, quantitative procedure of Meier (weight change of subcutaneously implanted cotton plugs) the effects of growth hormone (STH) and of cortisone upon granulation tissue were studied in rats. It was found that neither adrenalectomy nor hypophysectomy led to a diminished foreign body response to implanted cotton plugs saturated with saline alone. In rats of all three types (intact, hypophysectomized, adrenalectomized), 5 days after implantation the final weight of the pellet was diminished when it contained cortisone, but was increased when it contained STH. Quantitatively, the extent of the responses to the various hormone treatments was essentially the same in all three experimental groups. When both hormones were incorporated into the same cotton plug, the local depressive action of cortisone was reversed by the STH. The suppressive action of 0.75 mg. of locally administered cortisone was markedly counteracted by the addition of from 0.5 to 6.0 mg. of STH prepared by the Wilhelmi procedure. Control extracts prepared by the same method from a variety of tissues other than pituitary body proved devoid of such activity. Similarly, the local application of a highly purified ACTH preparation (corticotropin B), devoid of growth hormone activity, did not influence the amount of granulation tissue. Since adrenalectomized and hypophysectomized rats form normal amounts of granulation tissue, it appears that neither the adrenal gland nor the pituitary body is essential for the response to a foreign body. However, excessive amounts of cortisone or STH have profound influence upon granulation tissue formation. At present we are studying the local action of STH on the healing of wounds prepared according to a standardized procedure in the rabbit's ear. Preliminary findings indicate that the local administration of STH results in earlier closure of the wounds.

**THE RÔLE OF THE MAST CELL IN THE REACTION TO INJURY.** Earl P. Benditt (by invitation), Department of Pathology of the University of Chicago and the La Rabida-Jackson Park Sanatorium, Chicago, Ill.

*Abstract.* Mast cells have long been recognized in animal tissues, but their rôle in the physiology and pathology of the mammalian organism has been obscure. Anatomically they are intimately related to the blood vessels, particularly the small arteries, capillaries, and venules of areolar connective tissue. There is evidence now that in addition to containing heparin they also are associated with the histamine of the tissues. Our studies have been made principally on the rat which has a large complement of mast cells. Several facts have emerged from these experiments. The mast cells are very sensitive to mechanical damage and to damage by certain chemical agents of synthetic and of biologic origin. The damage is manifested acutely by spillage of the cell granules, histamine release, hyperemia, and protein-rich edema. Evidence of alteration in blood coagulation has been obtained. From this and other evidence to be presented we draw the following presumptive conclusions concerning the rôle of the mast cell in physiologic and pathologic states: regulated release of histamine from or by mast cells is an important factor in controlling vascular tone and permeability; regulated release of heparin and/or related substances controls local thrombus formation and, through the "clearing-factor" mechanism, controls lipid transport.

**CHRONIC POLYARTHRITIS IN RATS INJECTED WITH SPLEEN IN ADJUVANTS. H. C. Stoerk and (by invitation) T. C. Bielinski and T. Budzilovich, Merck Institute for Therapeutic Research, Rahway, N.J.**

*Abstract.* During a study of immunity against homologous tissue, it was observed that about half of 86 adult rats injected subcutaneously with suspensions or homogenates of cells from rat or beef spleen in Freund's adjuvant (heat-killed tubercle bacilli, mineral oil, and aquaphor) developed reddened, painful swellings of one or several joints. These arthritic lesions, which appeared 3 to 4 weeks after the injections, were not associated with conjunctivitis or urethritis. No pleuropneumonia-like organisms nor other pathogens could be grown from repeated cultures of heart's blood, spleen, or joints. Furthermore, no effect upon the development or the persistence of the arthritic lesions was observed when large doses of antibiotics (penicillin, streptomycin, aureomycin) were administered over several weeks. Groups of arthritic rats were killed at various intervals. When this abstract was submitted (2/10/54), some of the observations had extended over more than 5 months. Most lesions had persisted throughout this period. However, disappearance of some lesions followed by recurrence in the same or in other joints was observed not uncommonly. Histologically, the arthritic lesions were characterized by intense, predominantly chronic inflammation of the synovial tissues. Frequently these changes were associated with intense proliferation of the mesothelial lining cells. The joint cavities were free of fibrinopurulent exudate, but contained basophilic mucoid material mixed with moderately numerous acute and chronic inflammatory cells. There was no appreciable difference between lesions 4 weeks and 4 months of age, except for the development of fibrous ankylosis in some of the older lesions. Gross and microscopic examination of other organs failed to reveal significant changes. No endocardial or myocardial lesions were found. Attempts to explain the present findings include the possibility that splenic cells and synovial tissue have some "organ specific" antigen in common and that intense sensitization with this antigenic moiety leads to the development of the arthritic lesions. This possibility has its analogy in the demyelinating disease observed by Morgan and by Kabat and Wolf in monkeys injected with brain in Freund's adjuvant.

**TETRAZOLIUM STAINS FOR DIPHOSPHOPYRIDINE NUCLEOTIDE (DPN) DIAPHORASE AND TRIPHOSPHOPYRIDINE NUCLEOTIDE (TPN) DIAPHORASE IN ANIMAL TISSUES. Emmanuel Farber (by invitation), William H. Sternberg, and Charles E. Dunlap, Department of Pathology, Tulane University School of Medicine, New Orleans, La.**

*Abstract.* The oxidation of many metabolites is initiated by the action of dehydrogenases which require DPN or TPN as their active coenzymes. The  $H^+$  (and electrons) are passed along from the DPN or TPN through a series of oxidative enzymes including flavoproteins and cytochromes to react ultimately with molecular oxygen. The second step in this oxidative chain is believed to be catalyzed by flavoprotein reductases specific for either DPN or TPN. Normally, the oxidized reductases react with the cytochrome system but under artificial conditions they are capable of reacting with various dyes. When these reductases react with dyes, they are called diaphorases.

Many dyes, including methylene blue and certain of the tetrazolium salts, can be reduced by the diaphorases. When used as histochemical indicators, the reduced tetrazolium salt (formazan) precipitates as a colored deposit at sites of enzyme activity. We have developed tetrazolium staining methods which appear to be specific for the demonstration and localization of DPN and TPN diaphorases in tissues. Fortunately, routine frozen sections are found to retain insufficient concentrations of the dehydrogenase substrates and the coenzymes DPN and TPN to

permit significant spontaneous dehydrogenase activity, but do retain the diaphorases and effective amounts of many dehydrogenase apo-enzymes. Therefore, specific staining for DPN or TPN diaphorase may be obtained by supplying the appropriate substrates, coenzyme and cofactors.

For the demonstration of DPN diaphorase, frozen sections are incubated for from  $\frac{1}{2}$  to 2 hours in media containing a tetrazolium salt (blue tetrazolium or neotetrazolium), DPN, and substrates and cofactors for specific DPN-linked dehydrogenases. During the oxidation of the substrates by the dehydrogenases, DPN is reduced. The reduced DPN then serves as substrate for DPN diaphorase which in turn reduces the tetrazolium. The dehydrogenases therefore are not directly involved in the staining reaction and serve only to produce substrate for the action of the diaphorase. In similar fashion, the presence and localization of TPN diaphorase can be demonstrated by incubating frozen sections in media containing substrates for TPN-linked dehydrogenases together with TPN and essential cofactors.

In the rat kidney, the tetrazolium salts are deposited in patterns which are distinctly and reproducibly different for each of the diaphorases. These two patterns differ again from that observed in sections stained with tetrazolium for the succinic dehydrogenase system by the method of Seligman and co-workers.

These stains are applicable not only to the study of specific enzyme distributions but also can be used empirically to reveal histologic differences not demonstrable by conventional tissue stains. (See following abstract.)

**OBSERVATIONS ON THE HISTOCHEMICAL LOCALIZATION OF DPN AND TPN DIAPHORASES AND SUCCINIC DEHYDROGENASE SYSTEM IN THE RAT KIDNEY.** William H. Sternberg, Emmanuel Farber (by invitation), and Charles E. Dunlap, Department of Pathology, Tulane University School of Medicine, New Orleans, La.

*Abstract.* Following the development of methods for the histochemical localization of DPN and TPN diaphorases by the use of tetrazolium salts (see preceding communication) the usefulness of these methods was evaluated by the intensive study of a single organ, the kidney. Frozen sections of rat kidney were stained for DPN and TPN diaphorases as well as the succinic dehydrogenase system and compared with control sections stained by conventional methods.

Sections from the same kidney stained by the three enzyme methods have revealed striking zonal patterns and have provided stimulating insight into renal structure. Although in all three methods the brilliantly colored reduced tetrazolium (formazan) is the indicator of enzyme activity, each shows a highly reproducible and distinctively different zonal distribution in the cortex and medulla, and a corresponding regional distribution along the individual nephrons. With the use of the three enzyme methods, virtually all segments of the nephron can be sharply delineated and identified. In addition, segmental differences hitherto unsuspected have become apparent. For example, with succinic dehydrogenase the collecting tubules in the papilla are unstained, whereas with DPN diaphorase the collecting tubules are well stained to the very tip of the papilla. Cross-sections of the papilla and medulla stained for succinic dehydrogenase system indicate that the staining of collecting tubules begins abruptly at about the level where most of the ascending thin limbs of Henle become thick limbs. Thus a segmental difference in staining in the course of the collecting tubule has been found. Thin limbs of Henle are not distinguishable in sections stained for either the succinic dehydrogenase system or DPN diaphorase, but the thin limbs stain brilliantly with TPN diaphorase. They are especially well visualized in the outer medullary zone because the ascending thick limb of Henle (the predominant tubule in this zone) is less well stained with

TPN diaphorase and provides a good background for the intense blue staining of the thin limbs. This permits a topographic visualization of thin limbs which are notoriously difficult to identify by conventional methods.

The glomeruli, unstained with the succinic dehydrogenase method, show a delicate but definite staining with TPN diaphorase. Similarly, vascular endothelium and epithelium of the renal pelvis stain delicately with TPN and to a lesser extent with DPN diaphorase.

In the distal third of the rat papilla, staining for TPN diaphorase discloses in the interstitial tissue great numbers of stellate cells with nuclei and longer cytoplasmic processes oriented transversely to the direction of the collecting tubules. The significance of these cells has not been determined.

The rat kidney has a unique cortical zonal pattern quite unlike that of other mammalian kidneys studied. A broad inner zone is present which contains no glomeruli but consists for the most part of the distal portions of the proximal convoluted tubules. This zone forms wedge-shaped interdigitations with the outer glomerular cortex. With all three enzyme methods, the proximal convoluted tubules of the inner cortical zone stain differently from the proximal convoluted tubules in the outer cortical zone. There is considerable evidence that the segments of proximal convoluted tubules of the inner cortical zone are functionally different from the segments in the outer cortex. Finally, in preliminary studies with mercurial diuretics a selective inhibition of staining in the inner cortical zone has been observed with TPN diaphorase.

**BIOCHEMICAL AND HISTOCHEMICAL STUDIES OF IN VIVO AND IN VITRO NECROSIS OF LIVER TISSUE.\*** Robert E. Stowell and (by invitation) Max Berenbom and Peh-I Chang, Department of Pathology and Oncology, University of Kansas Medical School, Kansas City, Kans.

*Abstract.* The processes concerned with the death of cells, the most universal of all biologic phenomena, have received scant study by newer histochemical and chemical techniques. Pieces of mouse liver were incubated at 37° C. *in vivo* or implanted in the peritoneal cavity for varying periods up to 28 days. Cytologic, histochemical, and biochemical observations have been correlated at different intervals. Observations were made on pentose nucleic acid, desoxypentose nucleic acid, nitrogen, phosphorus, lipids, and water. The enzymes studied included acid and alkaline phosphatase, succinic dehydrogenase, cytochrome oxidase, esterase, and peptidase.

Oxidase enzymes decreased most rapidly during cell death produced in this manner, followed by esterases and phosphatases. The pentose nucleic acid decreased 93 per cent in 48 hours *in vitro*, as compared with 88 per cent *in vivo*. Desoxypentose nucleic acid disappeared more slowly. In some respects, the *in vitro* conditions were comparable to a poor tissue culture. The significance of the changes observed in tissue death under these conditions was discussed.

**THE EFFECTS OF REPEATED SMALL DOSES OF ETHIONINE ON THE PANCREAS, THE GROWTH, AND THE SERUM LEVEL OF METHIONINE OF RATS.** Walter R. Benson (by invitation) and James M. Young (by invitation), Departments of Pathology and Bacteriology, Duke University School of Medicine, Durham, N.C.

*Abstract.* Young rats given repeated intraperitoneal injections of small amounts of DL-ethionine showed considerable variation in rate of growth and in the degree

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and amount of degenerative changes in pancreatic acinar tissue. These observations indicated that ethionine, in addition to causing degeneration of pancreatic acinar tissue, may have a more generalized effect on body metabolism by decreasing the amount of available methionine. In order to evaluate these observations, the following experiment was performed.

Twenty-one white female rats weighing between 109 and 155 gm. were given daily intraperitoneal injections of a saline solution of DL-ethionine, 25 mg. per 100 gm. of body weight, for 12 days while on a stock diet with methionine in amounts adequate for normal growth. Ten animals had initial loss of weight followed by regular gain. Two animals maintained their weights. Six showed progressive weight loss or irregular loss and gain. Three were sacrificed early. The degenerative changes in the pancreas were greater in animals with weight loss. The acinar cells became atrophic with marked reduction in cytoplasmic volume and loss of peripheral basophilia and zymogen granules. After 1 week they were largely replaced by fatty tissue. The few acinar cells remaining had neither peripheral basophilia nor zymogen granules. In contrast to animals receiving large doses of ethionine, there was no significant fibrosis or inflammatory infiltration. The ducts were slightly dilated and persisted in the collapsed, atrophic pancreatic stroma. After injections were stopped, the animals gained weight rapidly and within 30 days were within the same range as untreated animals. Even in animals with severe weight loss, the pancreas had regenerated to almost normal gross and microscopic appearance. The reticulum framework, which was distorted and collapsed earlier, became more normal in configuration in areas of regeneration and was associated intimately with the vascular network.

Ten animals were given a second series of intraperitoneal injections for 18 days. Three maintained their weights. Two had initial loss and later gained in weight. Two had loss of weight occurring after 8 and 18 days of treatment. Three had irregular losses and gains. Degenerative changes in the pancreas were again more marked in animals with weight loss. Methionine levels determined by microbiologic assay revealed a mean level of 11.3 gamma per cc. of serum in 6 control animals, and a mean level of 6.5 gamma per cc. in 5 animals sacrificed immediately after the injections were discontinued.

These findings indicate a close relationship among pancreatic acinar function, serum methionine level, and rate of growth. Ethionine, in addition to exerting direct effects on cellular metabolism as an analogue of methionine, may have a more generalized effect on body growth by depressing the level of serum methionine.

**THE CONCENTRATION, DISTRIBUTION, AND EXCRETION OF RADIO-ETHIONINE ( $S^{35}$ ) IN THE RAT ON STOCK AND PROTEIN-DEPLETED DIETS—DETERMINED BY RADIOACTIVITY COUNTING AND RADIOAUTOGRAPHY.** Patrick J. Fitzgerald and (by invitation) Leon Hellman, J. Weinstein, and R. Schimmel, Pathology Department, State University of New York, College of Medicine at New York City, and the Department of Physics, Sloan-Kettering Institute, New York, N.Y.

**Abstract.** Male and female rats were given ethionine ( $S^{35}$ ) intraperitoneally. Organs and blood plasma were counted for radioactivity 30 minutes and 4, 48, and 96 hours after injection. Radioautographs were made of tissues removed at these periods. Just as with methionine—its antimetabolite—ethionine injection produced high concentrations of the isotope in the kidney, pancreas, gastro-intestinal tract, and skin. Significant values were present also in the testis and spleen. Radioautography showed localization of the isotope in the epithelium of the mucosa throughout the intestinal tract, in the hepatic cells, in the acinar cells of the pancreas (possibly in the zymogen granules), and in the hair follicles of the skin.



Stool and urine specimens collected at 48 and 96 hours after injection revealed that excretion was principally urinary and insignificantly fecal. Protein-depleted animals concentrated more isotope and generally at an earlier period than did animals on a stock diet.

**EFFECT OF ETHIONINE-INDUCED PANCREATIC DAMAGE ON IRON ABSORPTION.** Nathan Kaufman, Thomas D. Kinney, and Janis Klavins (by invitation), Cleveland City Hospital and Western Reserve University, Cleveland, Ohio.

**Abstract.** Male albino rats were fed *ad libitum* a purified synthetic diet containing 67 per cent glucose, 18 per cent vitamin-free casein, 4 per cent salt mixture, 11 per cent corn oil, as well as vitamin supplements. This is referred to as the basic diet. In the experimental groups 0.5 per cent DL-ethionine was added to the diet to induce pancreatic damage. Complete necropsies were done. The livers were stained for iron, and the amount of iron present was graded quantitatively from 0 to 5 plus.

Group I consisted of control rats fed the basic diet. The livers in these rats were graded 0 on histologic examination for iron. Another control group (group III) received the basic diet supplemented by 2 per cent iron citrate. The livers in these animals were also graded 0 for iron. Group II was fed the basic diet supplemented with 0.5 per cent DL-ethionine and there was definite pancreatic damage in all animals. The livers were graded 2 plus to 4 plus, with an average score of 3 plus, for iron. Group IV was fed the same diet as group II but with 2 per cent iron citrate added. Here again there was striking pancreatic damage and the histologic score for liver iron varied from 4 plus to 5 plus, with an average of 4.4 plus. This experiment included another group (group V) in which the intake of the basic diet was controlled so as to produce a weight loss essentially comparable with the weight loss in group II. In this starvation group, although the weight loss was 6.9 per cent as compared to 4.1 per cent in group II and the liver weight averaged 6.5 gm. as compared to 8.3 gm. in group II, the liver iron was not significantly higher than in the control groups.

The results indicate that pancreatic damage, as produced by intake of 0.5 per cent DL-ethionine introduced into the diet, is followed by increased absorption of iron from the gastro-intestinal tract with striking increase in the liver iron values.

**THE MORPHOLOGY OF ACCESSORY ADRENAL TISSUES IN THE TRANSITIONAL STAGE OF ADRENAL GLAND DEVELOPMENT.** James T. Hicks (by invitation) and Anderson Nettleship, Department of Pathology, University of Arkansas School of Medicine, Little Rock, Ark.

**Abstract.** Examinations of adrenal sections from 50 infants ranging in age from a 7 month fetus to 2.9 years showed periadrenal nodules composed of the following cell types: (1) outer zone cell type, (2) boundary zone cell type, (3) mixed 1 and 2 cell type, (4) tubular type, (5) medullary adult cell type, (6) paraganglionic embryonic cell type. There were 46 nodules of outer zone cell type observed in glands from children in this age range. The inner zone cell type was found in 25 glands, the mixed cell type in 25, the tubular type in 6, and the paraganglionic embryonic cell type in 3. This latter cell type is thought to be derived from neuroectodermal elements or perhaps it is related to either paraganglionic or medullary elements. Regardless of type, the nodules were found in extracapsular, capsular, and subcapsular areas. There appeared to be no predominance of any cell type in any particular area around the gland. The present work does not describe the early histogenesis and origin of these nodules, or their significance. Our observations tend to strengthen the views that the permanent cortex is derived from the migration and maturation of mesenchymal cells through the capsule. It therefore seems

reasonable to think that some of these cells do not complete their migration, but remain in the above areas, undergo further morphologic alteration, and form the basis for the resulting cortical adenomas found in one third to one half of all adults at necropsy. No attempt was made to differentiate cortical nodules and cortical adenomas in the present study.

**CYSTIC HYPERPLASIA OF ENDOMETRIUM AND BREAST IN MICE WITH  $I^{131}$  INDUCED PITUITARY ADENOMAS.** S. C. Sommers and (by invitation) R. N. Chute and A. S. Burt, Massachusetts Memorial Hospital, Boston, Mass.

*Abstract.* Development of endometrial hyperplasia and cystic mastitis occurred in a majority of the females among a group of 75 C-57 mice with induced so-called chromophobe pituitary adenomas after  $I^{131}$  therapy. Estrogenic effects upon vaginal epithelium were not found generally. Other tissue changes commonly observed included hyperplasia of brown pigmented, PAS-positive, ovarian stromal cells, subcapsular adrenal cortical proliferations of spindle cells, and enlargement or new formations of pancreatic islets. Male C-57 mice bearing similar pituitary tumors frequently showed cystic hypertrophy of prostatic glands and enlarged or newly formed pancreatic islets. About one third of the mice had some regenerated thyroid tissue 249 to 519 days after 300  $\mu$ c.  $I^{131}$  subcutaneously. These observations are interpreted as demonstrating that some so-called chromophobe pituitary adenomas produce a variety of trophic hormones.

**THE HYPOPHYSIS AFTER BILATERAL ADRENALECTOMY COMPARED WITH THAT IN SPONTANEOUS ADDISON'S DISEASE.** Agnes S. Burt (by invitation), Department of Pathology, Massachusetts General Hospital, Boston, Mass.

*Abstract.* In 6 patients with spontaneous Addison's disease the adenohypophysis contained numerous finely granulated, weakly Schiff-positive cells, while both normal basophils and acidophils were greatly reduced. These changes were much more striking in 3 of the patients who died in classic addisonian crisis than in the 3 who had received heavy desoxycorticosterone acetate therapy shortly before death. In 4 women bilaterally adrenalectomized for carcinoma of the breast and maintained thereafter on adequate cortisone therapy, differential counts of the anterior lobe approximated normal values. In a man adrenalectomized for carcinoma of the prostate, to whom adequate cortisone could not be given because of a psychosis, the differential count of the hypophysis approximated that in spontaneous addisonian crisis. This patient had developed brown pigmentation of the skin at the time of death. Both the hyperplasia of weakly Schiff-positive cells in patients with severe adrenal insufficiency and their reduction by adrenal steroid hormones suggest that these cells may be the source of ACTH in man.

**ABSENCE OF DEGENERATIVE CHANGES IN ARGENTAFFIN CELLS OF INTESTINAL MUCOSA OF COBALT-INJECTED GUINEA-PIGS.\*** W. Stanley Hartroft, Gerald A. Wrenshall (by invitation), and William D. Wilson (by invitation), Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

*Abstract.* De Duve *et al.* (1953) concluded that glucagon (HGF; hyperglycemic factor) is probably formed by the alpha cells of the pancreatic islets. He correlated the fact that it may be produced in some species by portions of the digestive tract with the presence of special argentaffin cells in the intestinal mucosa, "... since alpha cells also stain with silver." Gomori (1948) had previously emphasized that the argentaffin reaction is fundamentally different from all other silver impreg-

\* Supported in part by the National Research Council of Canada.



nations, and that misuse of the term argentaffin has resulted in considerable confusion. We have found that alpha cells of the adult male guinea-pig do not react positively to the argentaffin reaction (technique of Masson, 1914) although the enterochromaffin cells of the intestinal mucosa of this species do, thus confirming earlier findings of a similar nature by Lazowsky (1931) in the dog. Unfasted guinea-pigs injected subcutaneously with 0.50 cc. per kg. of a 6 per cent solution of cobalt chloride developed typical swelling, vacuolation, and degeneration of pancreatic alpha cells within 4 days. Enterochromaffin cells of the duodenum and upper jejunum of these animals were free of any degenerative changes and appeared identical with those in control guinea-pigs injected with 0.50 cc. per kg. of saline solution. Enterochromaffin cells of guinea-pigs' intestinal mucosa do not appear morphologically related to pancreatic alpha cells and, further, do not respond in the same way to injected cobalt. Consideration of these findings suggests that sources other than enterochromaffin cells be examined for the origin of glucagon in the intestinal tract of the guinea-pig and possibly of other species in which it has been isolated from this source.

**AN APPLICATION OF THE METHODS OF PAPER CHROMATOGRAPHY TO THE PROBLEMS OF GENERAL PATHOLOGY.** Stanfield Rogers (by invitation) and William M. Berton (by invitation), Department of Pathology, Duke University School of Medicine, Durham, N.C.

**Abstract.** Buzzati-Traverso (1953) reported differences in the chromatographic patterns of various strains of *Drosophila*. His method was to press the decapitated whole fly into chromatographic paper, develop the strips, and identify the components by routine chemical methods. It appeared to us that valuable data concerning the pathogenesis of disease might be obtained should tissues be studied similarly.

A spectrum of normal and diseased tissues was obtained by chromatography. One millimeter cubes of fresh tissue were boiled for 1 minute in distilled water, arresting enzymatic reactions. The fragments were pressed into sheets of Whatman No. 1 filter paper and dried. Ascending chromatograms were made. The solvents utilized included butanol-acetic acid, isoamyl alcohol- $\text{Na}_2\text{HPO}_4$ , and aqueous phenol. Both one and two dimensional chromatograms were made. Components which fluoresced in or absorbed ultraviolet light and those giving a positive reaction on application of ninhydrin were studied.

Various normal mouse organs were found to have specific chromatographic patterns. Those from renal cortex were regularly different from those from renal medulla. Heart muscle differed from voluntary muscle in both number and intensity of components. Lymph node and thymus were similar, although both differed markedly from the spleen. One of these differences appeared to be related to blood content of the specific organ. The effect of perfusion, however, was slight, even in the spleen, and was without influence in the chromatographic patterns of liver and kidney. On steeping tissues in a solution containing a known amino acid or pyrimidine, the chromatographic position of the added component was found to be identical with that of the added component without tissue.

As to age, the lymph nodes, spleen, and liver of newborn and immature animals differed from those of the mature. The chromatographic position of one of the components missing or diminished in the immature was similar to that usually identified with glutathione. On sulfhydryl identification little or no reaction was found in this area in young animals, while considerable reaction was found in adult material.

Both human and mouse tumors were studied. The chromatographic patterns of neoplastic tissue differed both qualitatively and quantitatively from the normal homologue. Lymphoblastic leukemic lymph nodes of mice had two components not

found in the normal. One of these components also appeared in the liver and spleen of the leukemic animals. Qualitative differences were found also on comparison of mouse breast cancer with hyperplastic lactating mouse breast. A human anaplastic carcinoma of the thyroid gland differed markedly from normal thyroid gland.

The livers of mice were studied 2 days following inoculation with Nelson's virus. Both qualitative and quantitative changes in the chromatographic patterns were noted. Efforts toward the identification of the substances responsible for the observed differences are under way.

**FALSE AND TRUE HYDATIDIFORM MOLE.** Nicholas M. Alter, Margaret Hague Maternity Hospital, Jersey City, N.J.

**Abstract.** Twenty to 50 per cent of the material from abortions, particularly in so-called missed abortion, shows molar changes on careful gross and microscopic examination. Microscopically, epithelial proliferation in the false mole is secondary to vascular changes. In true hydatidiform mole there is a primary neoplastic proliferation. True hydatidiform mole develops before villous circulation is established without development of the embryo in uniovular conception. True hydatidiform mole and a fetus are possible in binovular conception. Microscopically, in true mole the villous structures are avascular and have a periphery of neoplastic, primitive, embryonal, epithelial proliferation. The contrast in false molar structures is demonstrated by marked vascular changes and by differentiated epithelial hyperplasia due to regression of fetal circulation in the blighted ovum. Confusion between false and true hydatidiform mole results in contradictory statistics and wrong evaluation of treatment and prognosis of the patient.

**TELANGIECTATIC FIBROMYOSIS UTERI.** Nicholas M. Alter, Margaret Hague Maternity Hospital, Jersey City, N.J.

**Abstract.** The myometrium is often the site of diffuse histologic changes that are not as conspicuous as nodular forms. However, these changes eventually may become the source of intractable hemorrhagic conditions. These changes are partly congenital, partly acquired, and vascular in nature. They become conspicuous when they occur in rather extreme forms as illustrated by cases. Such telangiectatic lesions are well known to dermatologists, laryngologists, and internists (so-called angiomas and epistaxis). Advanced forms of vascular changes of the myometrium are known to gynecologists as fibrosis, sclerosis uteri, etc. This acquired form is individual in "aging of uterus." The process of arteriosclerosis in general shows individual variations. However, considering the vascular changes due to menstrual cycles and in pregnancy, the uterus obviously has to be regarded as one of the most active organs in women.

In obstetrics vague clinical terms are used, such as inertia and atony in hemorrhagic conditions. However, fibromyositis of the myometrium is the underlying condition leading to "muscular insufficiency." Other features may contribute to hemorrhagic conditions, such as blood dyscrasia. On the other hand, conspicuous small lesions may mask the essential basic defect, such as so-called placental polyp and small placenta accreta.

**MORPHOLOGIC ASPECTS OF THE TRANSITION FROM INTRA-EPITHELIAL TO INVASIVE CARCINOMA OF THE UTERINE CERVIX.** Robert H. Fennell, Jr. (by invitation), Department of Pathology, Massachusetts General Hospital, Boston, Mass.

**Abstract.** Carcinoma *in situ* of the uterine cervix becomes an invasive lesion in an unknown percentage of cases. Both the *in situ* and invasive lesion have been described extensively but scant information is available on the anatomy of the transition to the early invasive lesion. A group of cervixes diagnosed preoperatively as carcinoma *in situ* or invasive carcinoma have been studied by multiple blocks

and serial sections of areas that show invasion. It has been found that when invasion occurs in a cervix containing intra-epithelial carcinoma penetration beyond normal epithelial boundaries often occurs at multiple points. Indeed, early invasion may be evident at several hundred foci in the cervix. Furthermore, the cells of the penetrating nests of epithelium acquire more abundant and pinker cytoplasm than in the non-invasive areas and actual pearls may be seen. This is considered so characteristic that the presence of a pearl in an apparent *in situ* carcinoma makes a more thorough search for a focus of invasion necessary.

These findings are important in three respects. A readily recognizable change is described that facilitates the recognition of invasion. Secondly, the changes of early invasion are widespread so that the clinician can have confidence that his biopsy will be likely to detect the lesion that is of most concern to him. Thirdly, some of these cases were treated by simple total hysterectomy and patients now living and well indicate that small areas of invasion may not alter the prognosis. The finding of small areas of invasion in a hysterectomy specimen does not necessitate further treatment.

COMPARISON OF NUCLEAR SIZE AND NUCLEAR-CYTOPLASMIC RATIO IN INTRA-EPI-  
THELIAL AND INVASIVE CARCINOMA OF THE CERVIX UTERI. Alvan G. Foraker,  
Department of Pathology, University of Texas, M. D. Anderson Hospital for  
Cancer Research, Houston, Texas.

*Abstract.* Nuclear size and nuclear-cytoplasmic ratio, often mentioned as diagnostic criteria of cervical carcinoma, were analyzed by comparing planimeter measurements in basal, middle, and superficial layers of 25 cases each of intra-epithelial carcinoma, invasive carcinoma, and squamous metaplasia. Comparisons between the properties of nuclear measurements in different layers of the same epithelium were devised as "indices of maturation." The results showed:

1. Increased nuclear-cytoplasmic ratio was in general a more striking feature of pathologic squamous epithelium than increased mean nuclear size.

2. Intra-epithelial carcinoma in general manifested properties of mean nuclear size and nuclear-cytoplasmic ratio closely conforming to those of invasive carcinoma. Both types of carcinoma revealed greater mean nuclear size and nuclear-cytoplasmic ratio than metaplastic or normal epithelium.

3. Using diminution in nuclear-cytoplasmic ratio as the criterion, intra-epithelial carcinoma gave slightly more evidence of maturation through its layers than did invasive carcinoma. The similar properties of nuclear measurements of invasive and intra-epithelial carcinoma lend support to the concept that these two lesions are only different stages of a neoplastic process. The methods of measurement employed in this study could be utilized in quantitative comparison of nuclear size, nuclear-cytoplasmic ratio, and epithelial maturation as diagnostic criteria of "*in situ*" carcinoma in different laboratories.

EPITHELIAL ATYPICALITIES OF THE UTERINE CERVIX. James W. Reagan and Dorothy J. Hicks (by invitation), Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio.

*Abstract.* On the basis of our present knowledge the histopathologic diagnosis of carcinoma *in situ* should be restricted to lesions resembling those which have been demonstrated in the cervixes of women who later were proved to have outspoken cancer. Other lesions which do not meet the minimum requirements for carcinoma *in situ* but which are characterized by an immaturity of the component cells should be classified separately. These are not uncommon and are classified as atypical hyperplasia in this series.

This study is based on 102 cases of atypical hyperplasia represented by 143 "biopsies," 21 conized specimens, 23 complete uteri, and 3 amputated cervixes.

On the basis of cellular studies in some 10,000 women, the prevalence of atypical hyperplasia is about 0.77 per cent as compared with a prevalence of 0.38 per cent for carcinoma *in situ*. The lesions are more common in the Negro and are detected at an earlier age than in white women. The over-all mean age at detection was  $35.8 \pm 1.2$  years. Of the patients represented, 92 (90.2 per cent) were parous and in 17 the lesion was first demonstrated during pregnancy.

The lesions involve the cervical mucosa in the vicinity of the external cervical os or the portio vaginalis and may exist alone or in association with *in situ* or invasive cancer. The evidence accumulated to date indicates that the lesion may follow one of several courses: (1) the lesion may regress or cannot be demonstrated in subsequent biopsies, (2) the lesion may persist, (3) the change may be demonstrated in patients who are later proved to have carcinoma *in situ*, or (4) the lesion may be observed prior to the recognition of frank cancer. Selected cases illustrating the course of the lesions were shown.

**JUVENILE APONEUROTIC FIBROMA.** W. C. Thomas, Children's Hospital, Los Angeles, Calif.

*Abstract.* Fibromas developing during infancy and childhood form a group of clinical and histologic entities about which much speculation abounds. Attempts have been made to separate the distinctive syndromes so that a more rational approach may be made in recognizing the neoplasms and in directing proper therapy in individual cases. Dr. Louisa E. Keasbey introduced the subject in her paper on "Juvenile aponeurotic fibroma—a distinctive tumor arising in the palms and soles of young children" in 1953. We have encountered a morphologically similar case in the aponeurosis of the latissimus dorsi muscle in the lower trunk region. Speculations concerning the nature of this tumor were presented.

**THE SYNDROME OF INTESTINAL CARCINOID WITH MASSIVE HEPATIC METASTASES AND ENDOCARDIAL FIBROSIS WITH TRICUSPID AND PULMONIC STENOSIS: ITS RECOGNITION AND SIGNIFICANCE.** O. N. Rambo, Jr. (by invitation), Ira Gore, V. K. Vance (by invitation), and Harold Brown (by invitation), Veterans Administration Hospital and University of Utah College of Medicine, Salt Lake City, Utah.

*Abstract.* The coexistence of carcinoid tumors of the small intestine metastasizing to the liver and selective endocardial fibrosis of the right side of the heart appears to have significance beyond that of mere coincidence. Isler and Hedinger (1953) have analyzed necropsy material in their own hospital, as well as cases reported in the literature, and conclude that the occurrence of these lesions is far more frequent than would be expected by chance. Clinically this syndrome has a rather characteristic pattern; anatomically there are striking similarities in the few reported cases. Ante-mortem recognition of the syndrome has not been reported. Review of the literature indicates that examples of this syndrome have been published either because of unusual cardiac findings or as cases of carcinoid. In either instance, the other component of the syndrome has been referred to as an incidental finding. An illustrative case of ours is presented to bring attention to the syndrome and to stimulate interest in its ante-mortem diagnosis. Right-sided endocardial fibrosis with unusual deformities of the tricuspid and pulmonic valves and complete absence of lesions in the left heart suggest that the carcinoid tumor may have some effect on the endocardium. Ante-mortem diagnosis should not be difficult and biochemical studies of patients with this syndrome may prove valuable.

**JUVENILE XANTHOGRANULOMA (NEVOXANTHO-ENDOTHELIOMA).** Elson B. Helwig and Victor C. Hackney (by invitation), Armed Forces Institute of Pathology, Washington, D.C.

*Abstract.* Since Virchow's description, in 1871, of a child with cutaneous

xanthomas, occasional juvenile xanthomas with many features similar to those that occur in adults have been recorded. Another and different type of cutaneous xanthoma, however, has been observed in infants and children and has been designated nevoxantho-endothelioma by McDonagh. Fifty-three examples of the latter have been reviewed and the concept of nevoxantho-endothelioma critically evaluated. The lesions were about equally divided between the sexes, about one third were multiple, and nearly one fifth were present at birth. They ranged from a few millimeters to 1.5 cm. in diameter. The head and neck were the most common sites, but they occurred also on the trunk and extremities. The lesions regressed or were regressing after a few years, except in one infant who died. In that case they were distributed over the entire body, and at necropsy small lesions were found in one testis and in a lung.

Microscopically, the lesions usually involved the corium or sometimes the corium and the subcutaneous tissue. They were comprised of cells of spindle and polygonal shape, which often contained fat, in a groundwork of connective tissue. Large numbers of Touton giant cells were seen in many of the lesions. In no instance, even though serial sections were studied, could the endothelial giant cells described by others be distinguished. About one third of the lesions contained varying numbers of eosinophilic leukocytes. One or more cholesterol determinations of the blood were carried out on 11 patients and were normal in all. Lipid phosphorus determinations were made on four patients. The level was elevated on two occasions in the one patient who died; it was questionably elevated in another, and within normal limits in the remaining two. No objective evidence of xanthomatous disturbance of the skin had been noted by the parents of 43 patients. Partial or complete roentgen skeletal surveys of six patients showed no abnormalities.

The results of the lipid phosphorus determinations are inconclusive but indicate that more investigation should be made. The term nevoxantho-endothelioma is misleading and we suggest the descriptive term juvenile xanthogranuloma until the exact etiologic factors are known.

**MASSIVE ANGIOMATOUS TUMORS (PAPILLARY ANGIO-ENDOTHELIOMA IN VASCULAR HAMARTOMA) OF THE THORACIC WALL.** John B. Hazard, Cleveland Clinic, Cleveland, Ohio.

*Abstract.* Although angiomas are of common occurrence and may extensively involve skin areas, bulky vascular lesions are unusual. This presentation concerns a group of massive angiomatous tumors occurring in children and involving the soft tissues of the lateral upper half of the body, principally the thoracic wall. There were 3 patients; in 2 the tumor was present at birth and in the third it was noted 8 months later. The skin, subcutaneous tissue, muscle and deep fascia were involved. All the tumors were characterized by the presence of papillary masses of endothelial cells, partly filling angiomatous spaces, occurring in a hamartoma of mixed lymphangiomatous and hemangiomatous type. The endothelial cells were for the most part well differentiated and mitotic figures were rare. In 2 patients papillary neoplastic elements were demonstrated in regional lymph nodes. Despite this evidence of malignancy and the highly cellular nature of portions of the tumors, the patients were living without demonstrable metastases 3 to 5 years following partial or complete excision of the lesions. Such survivals are regarded as significant considering the usual rapidly malignant course of angiosarcoma. In order to distinguish these tumors from the more malignant and commonly recognized angiosarcoma, it is proposed that they be classified basically as papillary angio-endothelioma, with subsidiary specifications as to lymph node metastases and relation to hamartoma. The nomenclature of angiomatous neoplasms is discussed.

**INTERNAL MAMMARY LYMPH NODE INVOLVEMENT IN PRIMARY CARCINOMA OF BREAST: RADICAL MASTECTOMY STUDIES.** Mearl Stanton (by invitation) and



John P. Wyatt (by invitation), Department of Pathology, St. Louis University School of Medicine, St. Louis, Mo.

**Abstract.** For years the Halsted radical mastectomy with axillary lymph node dissection has been the surgeon's principal weapon of attack upon carcinoma of the breast. Anatomists have long stressed a second primary lymphatic reservoir: the internal mammary chain. Extension of the radical mastectomy to include the internal mammary lymph nodes has enabled the surgical pathologist to study the significance of this primary metastatic pathway. A study of 70 consecutive cases of "operable" breast carcinoma on which the radical mastectomy was combined with *en bloc* excision of the internal mammary lymph node chain is presented. Tumors of the subareolar area and all quadrants of the breast metastasized to the internal mammary chain. Internal mammary node metastases were present in approximately one third of the cases, irrespective of the occurrence of axillary involvement. This study supplies one reason for present-day dissatisfaction with the current surgical management of carcinoma of the breast.

**THE EFFECT OF CALCIUM AND OTHER CATIONS ON THE VISCOSITY OF THE CYTOPLASM OF EHRICH'S ASCITES TUMOR CELLS.** Edwin T. Nishimura (by invitation), Joseph A. Di Paolo (by invitation), and Willard T. Hill, Northwestern University Medical School, Chicago, Ill.

**Abstract.** The effect of certain cations on the viscosity of the cytoplasm of Ehrlich's ascites tumor cells was studied. The methods used were adapted from Heilbrunn and his collaborators. Their work on the eggs of marine annelids and sea urchin showed a definite relationship between the transfer of intracytoplasmic calcium ions and the viscosity of the cell cortex.

In the present experiments changes in the viscosity were measured by the movement of lipid granules by high speed centrifugation. The altered cells were examined with a phase contrast microscope. The addition of potassium oxalate, sodium citrate, or ethylene diamine tetra-acetic acid decreased the viscosity of the cytoplasm. The addition of calcium ions to washed tumor cell suspensions treated with the above agents prevented the reduction of the cytoplasmic viscosity. Similar results were noted with a high concentration of magnesium ions, but at a much lower level no influence was exerted. To demonstrate in each instance that the observed effects were occurring in viable cells, the treated tumor cells were injected into the peritoneal cavity of mice and were found to grow in the same manner as the untreated cells. It is concluded that calcium ions influence the viscosity of the cytoplasm of Ehrlich's ascites tumor cells and that magnesium ions show a similar effect only at a relatively high concentration. Sodium and potassium ions have no significant effect on the cytoplasmic viscosity under the conditions of this experiment.

**BRONCHIAL ADENOMA WITH DISTANT METASTASES.** Oscar H. Friedman (by invitation), Joseph Bellamy (by invitation), and Coleman B. Rabin, Columbia Memorial Hospital, Hudson, N.Y., and the Mount Sinai Hospital, New York, N.Y.

**Abstract.** There have been conflicting views as to whether the peculiar tumor known as bronchial adenoma is essentially benign or malignant. Of particular importance in consideration of this question is the occurrence of distant metastases in some of these growths. Evaluation of the significance of the metastases in relation to the malignancy of bronchial adenoma requires, as a basis, the determination of the frequency of the metastases, their nature, the evaluation of the influence of bronchoscopic biopsy on the occurrence of spread of the tumor, and, finally, the study of evidences of transformation of the primary growth from a benign to a

malignant neoplasm. To answer these questions, the literature on bronchial adenoma was reviewed and our own material was restudied. Cases of characteristic cylindroma were excluded because they present a separate problem.

In addition to those from the Mount Sinai Hospital, there are about 700 cases of bronchial adenoma in the literature. Of these, only 20 showed distant metastases. Several of the reports contain few or no details concerning the histologic structure of the growth. In only 7 was a post-mortem examination performed. In our own series of 79 bronchial adenomas there were 5 instances of distant metastases. All of the patients were examined at necropsy. Bronchoscopic biopsy could be excluded as a factor in the spread of the tumor. The low incidence of distant metastases indicates that the bronchial adenoma is either a benign tumor with a tendency toward malignant transformation in some cases, or is, in fact, a neoplasm of a low order of malignancy. This question might be answered by a comparison of biopsies in the earlier stages of the original tumor with those made when metastases occurred. Only a small amount of material could be studied from this point of view. The cases are presented by illustrations, and all of the material is discussed, particularly from the standpoint of the evidences of malignant transformation of the growth.

**PATHOLOGY OF TOTAL BODY IRRADIATION IN THE RHESUS MONKEY.\* HANS G.**

Schlumberger and Jacinto J. Vazquez (by invitation), Department of Pathology, College of Medicine, the Ohio State University, Columbus, Ohio.

**Abstract.** To determine the dose of total body irradiation lethal for 50 per cent of *Macacus rhesus* monkeys within 30 days of exposure (LD 50/30), 91 animals were treated with doses ranging from 300 r. to 900 r. The LD 50/30 was found to be approximately 550 r.

The gross and microscopic lesions are strikingly similar to those seen in man. The hematopoietic system is affected first, with destruction of the marrow and loss of lymphocytes from the lymph nodes and spleen. In animals that survive and are sacrificed several months after irradiation there is often a marked follicular hyperplasia in the spleen and lymph nodes. Hemorrhage occurs most often as petechiae in the skin, lungs, epicardium, stomach, and colon. Epilation was noted about 12 days after irradiation. Degenerative changes in the germinal epithelium of the ovaries and testes were observed following doses of 500 to 900 r. In the gastro-intestinal tract the colon is the site of hemorrhage and ulceration; however, 1 week after irradiation with 800 r. active epithelial proliferation may be seen in areas adjacent to foci of ulceration and necrosis. Necrotic gingivitis and oropharyngitis were found in 11 monkeys receiving 500 r. to 700 r. and surviving 2 to 3 weeks. They closely resemble that often observed in human casualties and begin in the region of the molar teeth as a shallow hemorrhagic ulceration. Involvement of the buccal mucosa follows and is accompanied by edema which may involve the entire face. A noma-like necrosis of the cheeks may be the end-result. Large ulcers along the lateral border of the tongue were observed in some cases.

**CYTOPLASMIC "INCLUSION BODIES" CONTAINING DESOXYRIBOGENUCLEIC ACID (DNA) IN CELLS OF HUMAN RECTAL POLYPS.† Cecile Leuchtenberger (by invitation), Institute of Pathology, Western Reserve University, Cleveland, Ohio.**

**Abstract.** In contrast to the universal presence of desoxyribonucleic acid (DNA) in all nuclei of all the cells of every living organism, the occurrence of

\* This article will appear in a subsequent issue of *The American Journal of Pathology*.

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DNA in the cytoplasm is rarely encountered and then in special circumstances only. In man cytoplasmic DNA has not been observed in cells of normal tissues but cytoplasmic inclusions containing DNA have been found in some virus diseases of the human. In this study evidence is presented of the constant occurrence of cytoplasmic inclusions containing DNA in the glandular epithelium of both benign and polypoid tumors of the human rectum. As far as we know such inclusions have not been reported heretofore. Cytoplasmic inclusions containing DNA were observed in all of the 63 human rectal polyps examined and were not found in 113 specimens comprising a variety of tissues with and without other pathologic lesions. The similarity of the "cytoplasmic inclusions" with viral inclusions and their possible significance for the question of etiology of polyps are discussed.

**THE APPLICATION OF AN INDUCED PULMONARY ARTERIAL COLLATERAL CIRCULATION AS POSSIBLE COLLATERAL BLOOD SUPPLY TO THE HEART.** William E. Bloomer (by invitation), Harold Stern (by invitation), and Averill A. Liebow, Departments of Surgery and Pathology, Yale University School of Medicine, New Haven, Conn.

*Abstract.* An attempt was made to establish alternative connections of the coronary arteries with the aorta, by way of the bronchial arteries. Expansion of the latter was induced by ligature of the left pulmonary artery. Symphysis of the pleura and epicardium was secured at the same operation by cauterizing the serous surfaces of the epicardium and pleura to be apposed with a silver nitrate stick, and suturing the medial surface of the left lung to the epicardium through a surgically created defect in the pericardial sac. In another series of dogs the procedure was the same, except that both the pulmonary arteries and veins were ligated, the latter in order to increase the resistance peripheral to the capillaries of the lung. In the former large precapillary anastomoses developed between the left coronary arteries and greatly expanded branches of the bronchial arteries. The direction of blood flow and the efficiency of these alternative connections of the coronary arteries with the aorta in protecting the myocardium after proximal interruption of the coronary arterial inflow remain to be determined.

**PATHOLOGIC FINDINGS IN NINE CHILDREN WITH "PRIMARY" PULMONARY HYPERTENSION.** Morgan Berthrong (by invitation) and Terence H. Cochran (by invitation), Glockner-Penrose Hospital, Colorado Springs, Colo., and University of Oregon Medical School, Portland, Ore. (From the Department of Pathology of the Johns Hopkins Hospital.)

*Abstract.* Pulmonary hypertension has long been recognized in patients with parenchymal diseases of the lungs, acquired heart disease—particularly mitral stenosis, and in many varieties of congenital heart disease. The morphologic changes in the pulmonary vessels of such cases often have been thought sufficiently widespread and severe to produce a mechanical obstruction to blood flow and thus to provide a reasonable explanation for the clinically demonstrated pulmonary hypertension. An occasional patient, without evidence of the above diseases, may die with pulmonary hypertension and show either widespread pulmonary thrombosis of the small arteries or obliterative pulmonary arteriosclerosis, also of the terminal arteries, as the apparent cause of the increased resistance to blood flow within the lesser circulation. In the past, cases with arteriosclerotic lesions have been considered, with but little justification, as examples of "Ayerza's disease." The vast majority of these cases have been in adults.

We have studied necropsies on 9 children who were from 1 to 9 years of age, who had been observed clinically to have had pulmonary hypertension sometimes from birth, who died as a direct result of cor pulmonale, and who were found to have as the apparent cause of that hypertension, vascular lesions of the small pul-

monary arterioles or arteries. In 4 cases, these lesions consisted of medial hypertrophy and endothelial proliferations of the tiniest pulmonary arterioles. The vessels with such changes lay in the walls of the alveoli and atria. The larger vessels showed only the effects of hypertension. Some endothelial changes suggested organized thrombi but the pathogenesis of these stenosing arteriolar lesions was otherwise obscure. The arterioles with medial hypertrophy resembled arterioles of the systemic circulation, a similarity never seen in the absence of cardiac or pulmonary disease in the lungs of infants older than 2 to 3 months. Such arterioles did resemble those of newborn infants. That these changes might be congenital malformations was discussed. In 4 other cases with identical clinical findings and of the same ages no such arteriolar lesions were seen; instead, severe intimal fibrosis or thrombi in different stages of organization were found in the medium-sized to small arteries immediately adjacent to bronchioles. It seemed quite possible that the fibrotic arteriosclerotic changes were the consequence of complete organization and recanalization of thrombi. The source of the thrombi, however, was not determined and the rarity of thrombo-embolic phenomena in infants and children was pointed out. One of these cases showed acute arteritis confined to the lungs and more severe than that which results from bland thrombosis. In another, old vascular lesions compatible with healed necrotizing arteritis were demonstrated. That there was a peculiar chronic necrotizing pulmonary arteritis in these 4 cases could be neither proved nor disproved. The final case exhibited both arteriolar and arterial lesions of the types described. In all but one case, the morphologic vascular lesions were thought sufficient to explain the persistence if not the cause of the hypertension. The possibility, however, of a primary functional increase of arteriolar resistance leading to pulmonary hypertension and thence to the vascular changes was considered. In 5 patients, persisting cyanosis was observed and in all of these the foramina ovals were anatomically patent though not of sufficient size to be functional in the absence of high right atrial pressures. In 3, cyanosis was not a clinical feature and in these the foramina were sealed. Polycythemia was present in all cyanotic patients as well as transiently so in one with a sealed foramen ovale. A close correlation between polycythemia and the pulmonary arterial thrombi was not established. Detailed clinical and pathologic findings in the 9 cases were presented with a review of the literature.

**PULMONARY PERIARTERITIS NODOSA: REPORT OF FIVE CASES.** Herbert Braunstein (by invitation), Cincinnati General Hospital, Cincinnati, Ohio.

*Abstract.* This report deals with 5 cases of necrotizing angitis with anatomical limitation to the lungs. The lesions meet the histologic criteria for classical periarteritis nodosa, and do not resemble those found in hypersensitivity states. Three of the cases had long-standing mitral stenosis and in one there was an absence of the interatrial septum. In the fifth there was severe pre-existing pulmonary arterial and arteriolar sclerosis of unknown etiology. All 5 cases thus had the common denominator of pulmonary hypertension. A brief discussion of the differentiation of this and other forms of necrotizing angitis was included. A review of the pertinent literature reveals the presence of similar cases previously reported. All presumably exhibited the common finding of pulmonary hypertension.

**PATHOLOGIC CHARACTERISTICS OF NECROTIZING PULMONARY ALVEOLITIS AS A MANIFESTATION OF HYPERSENSITIVITY AND ASSOCIATED WITH RECURRENT HEMOPTYSIS.** Jesse E. Edwards and (by invitation) Thomas W. Parkin and Howard B. Burchell, Sections of Pathologic Anatomy and of Medicine, Mayo Clinic, Rochester, Minn.

*Abstract.* In 7 cases in which recurrent hemoptysis was the chief symptom, the lungs showed structural alterations in the alveolar walls. The most striking change

was an acute necrotizing alveolitis. This was characterized by infiltration of cells, mainly neutrophilic granulocytes, in the alveolar walls and associated with fibrinoid necrosis of elements of the alveolar walls. Interruption of continuity of the alveolar membrane was readily demonstrated in sections stained for reticulum by Hortege's method. Thickening of the basement membrane by mucoid alteration of it was seen in all cases in varying degrees. Proliferation of alveolar lining cells was a prominent feature converting the lining membrane into a layer of readily identifiable cuboidal cells. Hemorrhage into alveolar spaces was a constant and striking feature. In 2 instances organization of blood in the alveolar spaces had progressed to a considerable degree, leading to fibrous obliteration of alveolar spaces. In only one of the cases was pulmonary arteritis observed. In 4 of the 7 cases lesions of periarteritis nodosa were observed in other organs. In each of the 7 cases renal lesions similar to those described by others and interpreted as resulting from hypersensitivity were seen. These varied from focal to diffuse glomerulitis, interstitial nephritis, tubular necrosis, and tubular hemorrhage.

The pulmonary alveolar lesions are interpreted as representing a hypersensitivity phenomenon on the basis of similarity to the lungs described in other reports on hypersensitivity, and because of the striking association between the pulmonary changes and the existence of nephritis and periarteritis nodosa.

**UREMIC PNEUMONITIS.** Howard C. Hopps and Robert W. Wissler, Schools of Medicine, University of Oklahoma, Oklahoma City, Okla., and University of Chicago, Chicago, Ill.

*Abstract.* The lungs of 107 persons who died with uremia have been studied in comparison with lungs from a control group of 429 who did not have uremia at the time of death. A detailed analysis of gross and microscopic findings indicates that uremic pneumonitis is a pathologic entity. These findings were enumerated and discussed. The occurrence of uremic pneumonitis was related to other (extrapulmonic) manifestations of uremia. In considering the pathogenesis of uremic pneumonitis, data were presented concerning the age and sex of the patient, the etiology of uremia, the duration of uremia, blood chemical changes, blood pressure, and whether or not oxygen therapy had been used.

**PNEUMOCONIOSIS FROM EXPOSURE TO KAOLIN DUST: KAOLINOSIS.** Kenneth M. Lynch and (by invitation) Forde A. McIver, Department of Pathology, Medical College of South Carolina, Charleston, S.C.

*Abstract.* Although the term "kaolinosiis" is defined as a kind of pneumoconiosis caused by inhaling particles of kaolin, recorded reports of its occurrence are sparse and such as are available fail to establish the condition fully in a position comparable to that accepted for certain other industrial dust diseases of the lungs. The purpose of this presentation is to report upon a necropsy study of kaolinosiis in the advanced or fatal stage, including x-ray and pathologic observations, as well as a brief discussion of the health and industrial implications.

**EFFECTS OF SILICATES ON THE RAT LUNG: AN EXPERIMENTAL STUDY.** M. David Orrahood (by invitation) and John P. Wyatt (by invitation), Department of Pathology, St. Louis University School of Medicine, St. Louis, Mo.

*Abstract.* The rôle of silicates in pneumoconiosis has undergone considerable revision in the last few years. These dusts were formerly considered inert, but recent investigation has shown that at least one silicate, diatomaceous earth, has been implicated in occupational lung disease in human beings. The following report is concerned with a temporal study over an 18-month period on the histopathologic effects of fullers' earth and diatomaceous earth in experimental animals. In addition, the effect of cortisone on these alterations is described. To ascertain the

purity of our sample, petrographic, polaroid, and chemical analysis, and x-ray diffraction studies of the diatomaceous earth were conducted prior to injection. Integrated morphologic and micro-incineration studies on the lungs were carried out. The conclusions are: (1) diatomaceous earth causes a progressive fibrous response which persists up to 18 months; (2) fullers' earth produces only a minimal connective tissue response, even after 18 months, with the anatomical collections of the dust being concentrated at the nodal points of the terminal bronchi; (3) cortisone had little effect on the connective tissue proliferation.

**ATYPICAL PROLIFERATIONS OF BRONCHIOLAR EPITHELIUM.** Lester S. King, Illinois Masonic Hospital Association, Chicago, Ill.

**Abstract.** In 1,450 consecutive necropsies 15 cases were found in which the lungs showed one or more foci of cellular proliferation of atypical character. The cell masses, which fill alveoli and occasionally seem to invade connective tissues, are of spindle or oat-cell type, generally uniform and well ordered, with occasional squamous change. These cells seem to be derived from the basal cells of terminal bronchioles or from the familiar cuboidal cells which grow down to line alveolar surfaces in zones of fixation. Predisposing factors to atypical proliferation include age (only 3 of the 15 patients were less than 70), circulatory disorders, the presence of infarction, localized fibrosis, or long-standing infection. These cell proliferations are considered essentially benign and reactive in nature, but their relation to carcinoma was discussed briefly.

**INFLUENCE OF BLOOD LIPID LEVELS ON INFLAMMATORY RESPONSE IN LUNG AND MUSCLE.\*** William Waddell (by invitation), Ronald C. Sniffen, and Lawrence L. Whytehead (by invitation), Massachusetts General Hospital, Boston, Mass., and the Memorial Hospital, Worcester, Mass.

**Abstract.** Idiopathic chronic interstitial pneumonitis with unusual deposits of endogenous lipid occurs in man. This report is concerned with the experimental production of a similar lesion in rabbits and with the influence of hyperlipemia on inflammatory reactions. Hypercholesterolemic rabbits were given intratracheal inoculations of a saline suspension of either *Klebsiella pneumoniae* or *Pasteurella pseudotuberculosis*. In addition, ischemia of the tibialis anticus muscles was produced by ligation of the major artery and vein and stripping of the muscle sheath. The findings were compared with those in a group of normocholesterolemic rabbits similarly treated.

Hypercholesterolemic rabbits infected with *K. pneumoniae* developed interstitial pneumonitis with many lipid-filled macrophages in the alveolar walls and spaces. Fat droplets were present in the capillary endothelial cells and arterial atheromas were particularly conspicuous in the zones of reaction. In hypercholesterolemic rabbits *Past. pseudotuberculosis* caused acute necrotizing pneumonitis with a surrounding reaction composed predominantly of lipophages. In normocholesterolemic rabbits these two organisms produced a far milder reaction with a predominance of macrophages, relatively few of which contained lipid. The pulmonary reactions in the hyperlipemic rabbits were similar to human pneumonias. Qualitatively similar lesions were produced in some stock-fed animals, suggesting that the lesions in the lipemic animals differed only quantitatively. The pneumonitis was more severe and widespread and the mortality much higher in hyperlipemic animals.

Ischemia in the muscles of rabbits resulted in acute necrosis with a neutrophilic infiltration followed by accumulation of macrophages and proliferation of endomysial fibroblasts. In hypercholesterolemic animals a great quantity of lipid accumulated in the macrophages, endomysial cells, and capillary endothelium. In stock-fed

\* This article will appear in a subsequent issue of *The American Journal of Pathology*.

animals very few lipid granules were present. The cholesterol and fat were always intracellular, indicating that disruption of the colloidal lipid system occurred after passage across the cell membrane. The accelerated formation of atheromas in the foci of pneumonitis, and the lipid in the capillary endothelium of infected lung and ischemic muscle of hyperlipemic animals suggested that local factors hasten the development of vascular lesions and that the physicochemical phenomena are the same as those which result in the gradual development of atherosclerosis. The variety of experimental procedures that have led to lipid deposition indicates that non-infectious physicochemical phenomena are responsible and that the problem is not one of general lipid metabolism, except as conditioned by high lipid levels. In all likelihood the factors concern not only the lipid, but the associated proteins responsible for stability of blood and tissue colloids.

**PULMONARY EMBOLISM: ITS INCIDENCE AND SIGNIFICANCE.** Abe Towbin (by invitation), Department of Pathology, Ohio State University, Columbus, Ohio.

*Abstract.* In the present study 132 cases of thrombo-embolic disease of the lung, occurring in 511 consecutive necropsies, were investigated. This study indicates that pulmonary embolism, though often undiagnosed clinically, is one of the most frequent causes of death.

In an institutional population composed largely of an older age group, necropsy was performed in 58 per cent of deaths. Thrombo-embolic lesions were present in the lung in 26 per cent of necropsies. In 14 per cent of cases massive embolism was the direct cause of death. Necropsy findings could be correlated with three clinical patterns: (1) Sudden death. In these cases, commonly regarded as examples of coronary thrombosis, necropsy generally revealed large coiled emboli loosely impacted in one or more main lobar arteries. This clinical pattern was observed in 18 per cent of the 132 cases. (2) Subacute form. These patients generally presented pulmonary symptoms for several days prior to death and were regularly diagnosed as bronchopneumonia. This group comprised 40 per cent of the cases with thrombo-embolic pulmonary disease. At necropsy, large occluded pulmonary arteries associated with recent infarcts were generally present. (3) Chronic form. Generally this type was incident to prolonged terminal illness. Almost always, arteries of small or medium caliber were affected; infarcts were frequently present. Respiratory distress was not a prominent feature clinically.

This study calls attention to two conditions, generally not realized, which predispose to the development of pulmonary embolism: (1) Upper respiratory tract infection. Individuals, particularly of the older group and who were treated with bed rest for minor respiratory disease, in many instances developed pulmonary embolism when ambulation was resumed. (2) Hypertension. In this study hypertension was present prior to the terminal illness in 45 per cent of the 132 cases which showed thrombo-embolic lesions in the lung at necropsy.

**SUDDEN DEATH DUE TO PULMONARY FAT EMBOLISM IN PERSONS WITH ALCOHOLIC FATTY LIVER.** Stanley H. Durlacher, J. Ralph Meier (by invitation), Russell S. Fisher, and William V. Lovitt, Jr. (by invitation), Louisiana State University School of Medicine, New Orleans, La., and University of Maryland School of Medicine, Baltimore, Md.

*Abstract.* Sudden death in cases of alcoholic fatty liver is well recognized, but the factors responsible for death are obscure. Recently W. S. Hartroft and J. H. Ridout demonstrated escape of lipid from fatty hepatic cysts into the biliary and vascular systems in experimental cirrhosis of the liver of rats suffering from choline deficiency. To determine whether fat embolism may be a factor in death due to fatty liver in man, 25 examples of alcoholic fatty liver were studied. Only cases without a history of trauma or findings of injury at necropsy were selected. Fat



stains were made of lung, kidney, brain, and liver. In 5 instances massive pulmonary fat embolism was found. The quantity of fat in the pulmonary vessels was as great or greater than that seen in acute deaths due to pulmonary fat embolism following fractures. In 3 other cases fat emboli were present in the pulmonary vessels in quantities that were not considered significant as a cause of death. Only rare vessels in the brains and kidneys were found to contain fat. It is concluded that death in some cases of alcoholic fatty liver without trauma is due to massive pulmonary fat embolism.

**FAT EMBOLI IN DIABETES MELLITUS.** Sidney P. Kent (by invitation), Department of Pathology, University of Alabama Medical Center, Birmingham, Ala.

*Abstract.* Fat emboli were first described in diabetic patients by Saunders and Hamilton in 1879. Their patients had also marked lipemia. Subsequently, several authors have reported fat emboli in diabetic patients. The emboli were thought by some to be derived from the lipemia which is common in diabetic patients; the small fat particles coalescing to form the emboli. Further, Lehman and Moore produced fat emboli in dogs by first causing them to develop lipemia and then administering ether. Does the lipemia which is commonly found in diabetic patients predispose them to the development of fat emboli? Graham, in reporting one case of fat embolism in a diabetic patient, found the emboli to be granular and therefore distinguishable from the fat emboli found in association with trauma. Is this granularity characteristic of the fat emboli found in diabetic patients?

The lungs of a selected series of 53 diabetic patients have been studied for fat emboli in an attempt to clarify the above questions. In order to eliminate trauma, in so far as is possible, as an etiologic agent, patients who had experienced trauma within 3 weeks of the time of death were not included. Cases with fat emboli were fairly common in both groups, somewhat more so in the diabetic group. However, most of the positive cases in both groups contained few fat emboli. A series of selected cases, non-traumatic and non-diabetic, were used as a control group.

**STUDIES ON THE ADRENAL ZONA GLOMERULOSA OF HYPERTENSIVE PATIENTS AND RATS, WITH SPECIAL REFERENCE TO THE EFFECT OF DIETARY SALT RESTRICTION.** Ernst Peschel (by invitation) and George J. Race (by invitation), Departments of Medicine and Pathology, Duke University School of Medicine, Durham, N.C.

*Abstract.* Participation of the adrenal cortex in various types of hypertensive disease is very probable, but the mechanisms are not clear. This work represents a controlled morphologic and histochemical study attempting to elucidate this problem, using adrenal glands from human necropsies and experimental animals. The zonae glomerulosae of the adrenal glands of 54 hypertensive patients who died while on the salt-free, rice diet were compared with those of 20 patients who died suddenly without preceding illness, of 13 hypertensive patients on normal diet, and of 15 malnourished cancer patients. Due to advanced renal insufficiency, serum electrolyte disturbance, including hyponatremia, existed terminally in 35 of the 54 patients on the rice diet. The width of the zona glomerulosa of the patients on the rice diet was found to be increased significantly by statistic methods in 24 per cent of the cases. It was within the normal range in 70 per cent, and insignificantly below the normal range in 6 per cent. Hypertensive rats on salt-free diet compared to hypertensive rats on normal diet showed a marked increase in width of the zona glomerulosa. Similarly, normal rats on the salt-free diet compared to normal rats on diets with normal salt content showed a marked increase in width of the zona glomerulosa. The latter observation is in agreement with those of several other authors. The cytoplasmic fat content was decreased in the human adrenal glands when the width was greater than normal, and was the same as in

the controls, when the width was in the normal range. Special histochemical staining techniques identified the cytoplasmic fat as being ketosteroid in type. No conclusions can be drawn regarding the rôle of the adrenal cortex in the genesis of hypertension. In regard to the influence of the rice diet on the adrenal cortex, definite cortical atrophy, such as would indicate reduced cortical activity, was not observed in any instance. The increased width and decreased fat content of the zona glomerulosa are interpreted as evidence of increased hormone production without storage. It is suggested that this may be due to the decreased sodium: potassium ratio which stimulates the production of electrolyte-regulating corticoids.

**HEMATIN-LIKE PIGMENT IN FRESH KIDNEY HOMOGENATES FROM RABBITS WITH HEMOGLOBINURIC NEPHROSIS.** Joseph J. Lulich, Department of Pathology, University of Wisconsin Medical School, Madison, Wis.

*Abstract.* Mallory has indicated that fatal uremia never develops in patients treated for shock in the absence of pigment nephropathy. Due to the apparent relationship between pigment retention and renal failure, it seemed desirable to determine the concentration and chemical composition of the brown pigment, the ratio of the pigment and protein present in the kidneys, and the relative importance of edema versus protein retention on the production of enlarged kidneys. Information pertaining to these questions was secured by the following analyses.

Segments of one kidney were saved for microscopic examination, subjected to air drying, and analyzed for N following Kjeldahl digestion. The other kidney was suspended in a water-saponin solution and homogenized for 60 seconds. Following centrifugation the water-insoluble brown pigment was extracted with alkaline pyridine. After the second centrifugation the concentration of oxyhemoglobin or its derivatives in the different extracts was established by determining the pyridine hemochromogen concentration at 540 m $\mu$  in a Beckman spectrophotometer. The pigment extracted from the kidneys was dissolved in alkaline pyridine solution at an equivalent concentration to commercially prepared crystalline hematin. Spectrophotometric absorption studies indicated that the extracted pigment resembled, but was not identical with, crystalline hematin. Iron concentrations also were determined in some of the extracts and the insoluble homogenized kidney residues. There is an excellent correlation between the retention of brown pigment and renal failure. Increases in kidney weight may be due, for the most part, either to a retention of protein and/or water. Iron does not appear to be the principal toxic factor because only minimal concentrations of iron were found in the kidneys. The protein pigment ratios varied widely in the different kidneys.

**BILIRUBIN-LIKE CRYSTALS IN CASES OF ERYTHROBLASTOSIS FETALIS.** Harlan I. Firminger and Lauren R. Moriarty (by invitation), Department of Pathology and Oncology, Kansas University Medical Center, Kansas City, Kans.

*Abstract.* In a previous study of frozen sections of adrenal glands from 320 necropsies, small yellow-brown birefringent rhomboid crystals resembling bilirubin were found only in each of 4 cases of erythroblastosis fetalis. In the present study similar crystals were found in 16 of 18 cases of erythroblastosis fetalis. Out of 114 selected control cases these crystals were found in only 4 and in each instance they were in areas of old hemorrhage. Crystals were found most commonly in the adrenal glands, liver, kidney, spleen, and various other organs in order of decreasing frequency. They were found in fresh unfixed adrenal gland 2 hours after death, and, in the only case in which the peripheral blood was examined post mortem, crystals were found in cells of the myeloid series. Wright's stain of dried smears did not remove the crystals, but chloroform, xylol, or benzene used for paraffin embedding completely extracted them from the tissues. Almost identical crystals have been produced by injection of homologous blood subcutaneously in mice, but



Dunn and others have shown that a minimum of 7 days is required for the development of these crystals *in vivo*. All of the cases of erythroblastosis in this series died within 5 days after birth, suggesting that hemolysis *in utero* accounts for the excessive erythropoietic activity and the increase in erythroblasts in the peripheral blood so characteristic of these infants even before they develop jaundice as the result of the accelerated hemolytic process soon after birth.

The presence of these crystals in newborn infants would seem diagnostic of erythroblastosis fetalis if sections do not include areas of old hemorrhage. Also search of peripheral blood for these crystals in thick smears may be a valuable clinical diagnostic tool. Further search for these crystals is under way in clinical cases with excessive hemolysis.

**CARDIAC LESIONS PRODUCED EXPERIMENTALLY IN ANIMALS GIVEN CRYSTALLINE STREPTOCOCCAL PROTEINASE INTRAVENOUSLY.** Aaron Kellner and Theodore Robertson, Department of Pathology, the New York Hospital-Cornell Medical Center, New York, N.Y.

*Abstract.* Striking focal necrosis of the myocardium has developed in rabbits, mice, and guinea-pigs given a single intravenous injection of a solution of crystalline streptococcal proteinase, a papain-like proteolytic enzyme isolated from filtrates of group A hemolytic streptococci. The lesion was present in 10 of 13 rabbits given 1.5 to 2.0 mg. of the enzyme per kg., in 22 of 36 mice given 0.5 to 1.0 mg. per animal, and in 4 of 8 guinea-pigs given 1.0 to 1.5 mg. per animal. In addition, small verrucous areas of inflammation of the heart valve leaflets were observed in 2 rabbits and 4 mice following such injections. No similar lesions of the heart muscle or valves were encountered in numerous control animals studied.

The myocardial lesions were first evident about 12 hours after injection and consisted of focal areas, often widespread, in which the muscle fibers had lost their cross-striations and were eosinophilic, swollen, and granular. A sparse infiltration of polymorphonuclear leukocytes was present in some of these areas. Twenty-four to 48 hours after injection the affected muscle fibers had undergone complete necrosis and there was a moderate infiltration of inflammatory cells in which histiocytes and lymphocytes predominated. Proliferating muscle cell nuclei were present within and about the necrotic areas. In mice, calcification of the foci of cardiac muscle necrosis was a particularly prominent feature; this was often present as early as 48 hours after injection and was clearly visible to the naked eye as yellow streaks and plaques on the epicardial surface. The myocardial lesions appeared in general to have a random distribution. They were present in both ventricles, in the papillary muscles, and occasionally also in the auricles. The lesions bore no relation to blood vessels, which were patent and morphologically unchanged. It is noteworthy that, with the exception of a few areas of necrosis of skeletal muscle in rabbits, no other visceral lesions attributable to the injected streptococcal proteinase were seen in these animals.

The findings are of particular interest because of the association between rheumatic fever and antecedent streptococcal infection. They suggest that specific streptococcal products may be directly implicated in the pathogenesis of the anatomical changes present in rheumatic heart disease.

**RHEUMATIC FEVER-LIKE LESIONS IN THE GUINEA-PIG: CORRELATION OF PATHOGENIC, ANAPHYLACTOGENIC, AND CHEMICAL PROPERTIES OF CERTAIN MUCO-POLYSACCHARIDES OF KLEBSIELLA PNEUMONIAE TYPE B27.** Russell S. Jones and (by invitation) Yolande Carter and James deW. Rankin, University of Oregon Medical School, Portland, Ore.

*Abstract.* This study was directed primarily toward the rôle of hypersensitivity as the pathogenetic mechanism in the rheumatic fever-like lesions of the guinea-pig.

Polysaccharides were derived from the Friedländer type B27 organism by four different techniques: one employed formamide, two used alkaline hydrolysis, and one an initial alcohol precipitation followed by acetic acid hydrolysis. The criterion of hypersensitivity was the elicitation of anaphylaxis. The various polysaccharides were tested for anaphylactogenic properties by subcutaneous injection in 1 mg. quantities for 5 days; on the 14th day variable quantities (0.1 to 10.0 mg.) of the substance were introduced into the ear veins. Only the acetic acid-derived polysaccharide produced sensitization; none of the other polysaccharides would elicit anaphylaxis in animals sensitized with the acetic acid-derived polysaccharide. The latter produced mild anaphylaxis in animals sensitized with killed bacteria.

Chemical properties were based upon determination of the total nitrogen, hexosamine, hexuronic acid, acetyl content, and types of sugar by paper chromatography. Two of the polysaccharides were selected for a study of the pathogenic properties, one derived by alkaline hydrolysis, and the other with anaphylactogenic properties, the acetic acid hydrolysis polysaccharide. Chemically, their major difference appears to be in acetyl content. The acid hydrolysis polysaccharide is the more toxic with an intravenous  $L_{50}$  of approximately 20 mg. per kg. The delayed effects of this polysaccharide in the guinea-pig are comparable to the "toxic property" of gram-negative bacteria.

The effect of subcutaneous and intravenous routes was investigated. Twenty guinea-pigs weighing 200 to 300 gm. were given daily 5 mg. of the alkaline hydrolysis polysaccharide subcutaneously; a similar group was given 5 mg. per day of the acid hydrolysis polysaccharide; animals in both groups were sacrificed at 3, 7, 14, 21, and 28 days. After daily intravenous (ear vein) injections of these two polysaccharides in 2.5 mg. quantities, animals were sacrificed at 3, 7, and 14 days. Proliferation of endothelial and stromal fibroblastic cells was prominent in aortic and mitral valves but the ground substance showed only moderate change. "Aschoff-like bodies" were found about the coronary arteries.

Previous studies have shown that such lesions are non-specific in respect to the inciting agent. Certain observations in the present experiment cast doubt upon the specificity and significance of anaphylactic hypersensitivity as the pathogenetic mechanism in the guinea-pig lesions: (a) the non-sensitizing polysaccharide produced severe lesions, (b) the lesions appeared by the third day of injection.

**OBSERVATIONS ON INTIMAL REPAIR FOLLOWING EXPERIMENTALLY INDUCED TRAUMA TO THE RABBIT AORTA.** John T. Prior and Robert V. P. Hutter (by invitation), State University of New York Upstate Medical Center, Syracuse, N.Y.

*Abstract.* The pathogenesis of the atherosclerotic plaque in man is a process concerning which relatively little is known. Present-day knowledge has been founded upon a succession of theories including a simple inflammatory basis, physicochemical changes within the intimal extracellular colloids, aberrations in serum lipids and lipoproteins, and more recently a concept that the atheroma may be the sequel to simple intimal thrombus formation. Much of this existing confusion appears to be due to overemphasis upon the rôle and manner of lipid deposition in a variety of experimental animals, while the more basic processes of injury and repair within the subendothelial zone have largely been ignored.

The present study is an attempt to confirm Duguid's contention (1948) that intimal thrombi and their subsequent organization and degenerative changes may produce mural masses indistinguishable from human atherosclerotic plaques. A series of 30 rabbits was subjected to extensive intimal trauma of the lumbar aorta with a blunt needle, and the animals were sacrificed at intervals from 3 hours to 200 days for serial study of injury and repair. A lesion grossly resembling the human plaque was observed at 35 days. This was composed of subendothelial fibroblastic cells and large amounts of elastic tissue. This lesion was histologically

identical with intimal fibro-elastic thickenings described in children by Prior and Jones (1952), which they postulated might be the earliest phase in the development of the adult lesion. Although lipid material and macrophages were never a prominent feature of this experimentally produced lesion, calcification was noted as early as 49 days, but its location and appearance differed somewhat from that in the human lesion. The morphologic changes produced in this experiment are contrasted with the results of other artificially induced vascular trauma (thermal, bacterial, and various chemicals). While our observations are not in complete harmony with Duguid's findings, we have found intimal fibro-elastic plaques identical with those observed in infants and which we contend may be the earliest phase in the development of the adult atherosclerotic plaque.

**POST-MORTEM STUDIES ON CORONARY ATHEROSCLEROSIS AND SERUM BETA LIPO-PROTEINS.** David M. Spain and (by invitation) Victoria A. Bradess and Irving J. Greenblatt, Beth-El Hospital, Brooklyn, N.Y., and Grasslands Hospital, Valhalla, N.Y.

*Abstract.* To determine more precisely the relationship between serum beta lipoproteins and atherosclerosis of the coronary arteries and aorta, post-mortem evaluation of the degree of coronary and aortic atherosclerosis was correlated with the pattern of the beta lipoproteins in the post-mortem blood. Prior to this it was determined that the post-mortem serum beta lipoprotein determinations reflected quite accurately those made during life. One hundred consecutive sudden deaths (homicide, suicide, accident, coronary occlusions, etc.) were studied. The cases were also classified according to body types (mesomorphs, ectomorphs, endomorphs) with the obvious limitations imposed by doing this post mortem. The findings indicated a close correlation between the degree of coronary atherosclerosis and the serum beta lipoprotein pattern. Close correlation is most accurately reflected in females and in the mesomorphic males. The ectomorphic males studied in this group revealed no significant relationship between the degree of coronary atherosclerosis and the serum beta lipoprotein pattern.

**CORONARY ARTERY DISEASE IN INFANCY.** W. C. Thomas, Children's Hospital, Los Angeles, Calif.

*Abstract.* Cases of coronary artery disease in infancy are reviewed. Evidences of clinical involvement included symptoms of cardiac impairment and signs of cardiac enlargement with failure. Electrocardiographic evidence of myocardial infarction was seen in one of the cases. Diffuse prominence of tortuous coronary arteries associated with focal areas of dilatation were seen. Recent thrombi were present. There were infarctions of the myocardium. Theories as to the origin of infantile disease of the coronary arteries are given.

**CARDIOVASCULAR AND SMOOTH MUSCLE LESIONS IN THE COURSE OF EXPERIMENTAL NEPHROPATHY.\*** Jacob Churg and David Lehr (by invitation), Barnert Memorial Hospital, Paterson, N.J., and New York Medical College, Flower and Fifth Avenue Hospitals, New York, N.Y.

*Abstract.* Severe nephropathy can be induced in rats and other animals by a single injection of poorly soluble sulfonamide compounds, such as acetyl sulfathiazole. After a brief period of urinary suppression, prolonged polyuria and hypostenuria set in. Blood pressure rises to hypertensive levels. The renal damage is

\* This investigation was supported by a research grant (H-890) from the National Heart Institute, the National Institutes of Health, Public Health Service.

accompanied by marked changes in the myocardium and in the smooth muscle of the arteries and gastro-intestinal and urinary tracts.

In the rat, the most prominent arterial changes are seen in the aorta and its major branches, and to a lesser extent, in the large pulmonary arteries. About 4 to 6 days after an intraperitoneal injection, degenerative changes appear in the smooth muscle fibers of the media. These consist, in rapid succession, of hydropic degeneration, fragmentation, loss of nuclei, and dissolution of myofibrils. Elastic fibers in the affected media become basophilic and may break up. Deposition of calcium follows. In areas of most severe damage, there is swelling of the degenerated media due to deposition of connective tissue ground substance and proliferation of fibroblasts. When calcification sets in, these areas give the aorta a "bamboo stick" or "goose neck" appearance. The entire process induces little or no inflammatory response. Occasionally, a more severe change, with complete loss of architecture, "fibrinoid" transformation, and marked polymorphonuclear exudation, can be observed in a small segment or at the origin of the intercostal or coronary arteries.

The medium-sized and small arteries, particularly those of the coronary system and the gastro-intestinal tract, show similar, though perhaps less intense changes. Degeneration of muscle fibers does not always proceed to necrosis and evidence of regeneration can be seen early. Inflammatory reaction and subsequent perivascular fibrosis are more prominent and necrotizing "fibrinoid" arteritis is not infrequent.

The heart exhibits early changes, beginning 24 hours after the injection. Small collections of mononuclear cells with admixture of polymorphonuclear leukocytes appear in the myocardium and occasionally in the valves. About the 4th day small areas of degeneration can be seen, involving a segment of a single muscle fiber or a few adjoining fibers. These become more eosinophilic, stain red with the periodic acid-Schiff reagent, myofibrils and nuclei disappear, and finally calcium is deposited. There is no accompanying inflammation, though mononuclear cells may be scattered in the connective tissue septa. In other instances the myocardium shows large areas of necrosis with an acute inflammatory response.

Changes in the muscle of the gastro-intestinal tract, particularly the stomach, and of the urinary bladder, consist of swelling, fusion, increased acidophilia, and finally loss of nuclei and myofibrils. Subsequent calcification may be pronounced. With less severe damage, regeneration of muscle fibers is seen.

These findings suggest that renal damage induces an abnormal state in the smooth muscles of the body, and perhaps also in the cardiac muscle. Where muscle fibers are subjected to stress and strain, degeneration and necrosis occur. Under certain circumstances, necrosis is accompanied by severe inflammatory reaction and the appearance of abnormal protein material ("fibrinoid" or fibrin) in the necrotic areas.

**A MICROSPECTROGRAPHIC STUDY OF THE ARTERIOLES IN BENIGN AND MALIGNANT HYPERTENSION.** P. O'B. Montgomery and E. E. Muirhead, Department of Pathology, Southwestern Medical School of the University of Texas, Dallas, Texas.

*Abstract.* The microspectrographic absorption characteristics of arterioles in the normal and various pathologic states were presented by means of graphs and photomicrographs. These data included the absorption characteristics of (1) normal human and dog arterioles; (2) acutely necrotic arterioles from the bilaterally nephrectomized dog and from the human with malignant hypertension; (3) arterioles showing hyaline arteriolar sclerosis from patients with benign hypertensive cardiovascular disease and one patient with benign hypertensive cardiovascular

disease and diabetes with diabetic nodular glomerular sclerosis. The absorption characteristics of these various arterioles were compared at 50 Å intervals from 2500 Å to 3600 Å. Comparison of these data indicates that the acute arteriolar necrosis of malignant hypertension and the hyaline arteriolar sclerosis of benign hypertension have the same microspectrographic characteristics as normal arterioles. These observations are interpreted as another link in the evidence to support the concept that the arteriolar lesions of benign and malignant hypertension are the consequences of alterations of the smooth muscle of the arteriolar wall.

THE EFFECT OF FLUID AND ELECTROLYTE IN BILATERALLY NEPHRECTOMIZED DOGS.

J. L. Orbison and (by invitation) Evelyn R. Peters and C. L. Christian, Institute of Pathology, Western Reserve University, Cleveland, Ohio.

*Abstract.* Many investigators have found that untreated bilaterally nephrectomized dogs rarely develop hypertension or vascular lesions. It has been previously shown by the authors that the intraperitoneal injection of 100 cc. per kg. of 0.85 per cent NaCl or of an "isotonic balanced salt solution" will induce both hypertension and vascular necrosis in such animals.

In an attempt to clarify the relative rôle of fluid and electrolyte in this process, one group of bilaterally nephrectomized dogs were given small amounts of 10 per cent NaCl intraperitoneally, and another group the same amount of equimolar NaCl and  $\text{NaHCO}_3$  in 10 per cent solution intraperitoneally. Both groups developed hypertension of similar degree, but only the dogs given NaCl and  $\text{NaHCO}_3$  developed a significant amount of arteriolar necrosis. In view of this additional evidence of the dependency of vascular necrosis on electrolyte composition, one group of bilaterally nephrectomized dogs was given an "isotonic solution of balanced salt," and another group an isotonic solution composed of the cations used in the balanced salt, but using only the chloride salts. Both groups developed hypertension and arteriolar necrosis, but the group given balanced salt had greater hypertension and more severe arteriolar necrosis.

Since 11 of the 23 dogs given 100 cc. of fluid per kg. increased their blood volume less than 10 per cent of control values, blood volume does not appear to be a critical factor in these observations. Since all animals, even those given hypertonic solutions, had some increase in extracellular fluid, this cannot be eliminated as a factor in the development of hypertension. However, the comparison of dogs given the chloride salt of cations with those given bicarbonate or mixed anion salts of cations suggests that the intensity of arteriolar necrosis and the hypertension is in part anion-dependent.

THE MYOID NATURE OF THE CELLS COVERING THE HUMAN RENAL GLOMERULUS.

J. F. A. McManus, University of Alabama Medical Center, Birmingham, Ala., and the Mount Desert Island Biological Laboratory, Salisbury Cove, Me.

*Abstract.* Electron microscope studies by Peace and Baker, Rinehart, Dalton, Hall, and others have shown a specialized structure in the cells covering the renal glomerulus. As Hall has recalled, Gruenwald and Popper described a loss of the epithelial cells covering the glomerulus in early neonatal life. These data have suggested reconsideration of the cells covering the human renal glomerulus, the so-called visceral layer of Bowman's capsule. In several varieties of fishes, e.g., the dogfish, it can be seen that the cells covering the renal glomerulus are continuous with the cells of the renal arterioles. Only rarely can such a relationship be made out in man. The concentration of the enzyme, 5-nucleotidase, in the cells covering the glomerular capillaries in early ischemia has already been described by McManus and Lupton. This enzyme is present most characteristically in smooth muscle cells. Epithelial



crescent formation, as in subacute and chronic glomerulonephritis, is not associated with any excess of 5-nucleotidase in the cells making up the crescent. These data and others make it highly probable that the cells covering the human renal glomerulus in adult life are myoid in type.

**GLOMERULAR LESIONS IN RATS WITH CHRONIC HYPERTENSION.** Simon Koletsky, Institute of Pathology, Western Reserve University, Cleveland, Ohio.

*Abstract.* Chronic hypertension was produced in rats by two different techniques: (1) temporary interruption of circulation to one kidney and subsequent reaction of the opposite kidney, and (2) ligation of renal artery branches. Both methods involved substantial reduction in renal substance. The hypertension usually developed within a few weeks after the experimental procedure and then existed for a variable period, usually from a few weeks to several months, in the presence of adequate renal function and without significant lesions in the kidney remnant. Thereafter, there was progressive pathologic destruction of glomeruli which resulted in impaired renal function. The glomerular involvement appeared to be cyclic in nature so that at various intervals of sacrifice the kidney generally showed lesions in all stages of development, from slight to advanced. Eventually there was obliteration of sufficient glomeruli to cause death in uremia. The glomerular lesions were varied and consisted of progressive fibrosis and hyalinization of tufts, capsular adhesions, alternative change with fibrinoid necrosis and fat deposit, and epithelial proliferation with crescent formation.

Several factors have been considered in the pathogenesis of the renal lesions. Our studies afford little or no evidence that they are secondary to intrarenal arteriolar vascular disease or to complicating infection such as pyelonephritis. There is the theoretic possibility that the glomerular changes represent a form of overwork decompensation in a kidney attempting to maintain function with a significantly reduced renal mass. So far it has not been possible to obtain supporting evidence for such a hypothesis. In this study the development of the lesions appeared to depend, either directly or in some indirect way, on the existence of chronic hypertension.

## SYMPOSIUM ON DISEASES OF THE NERVOUS SYSTEM AND OF THE NEUROMUSCULAR APPARATUS

Referee (by invitation of the Council): Harry M. Zimmerman

**MIXED TUMORS OF THE BRAIN—CONJOINED GLIOBLASTOMA MULTIFORME AND SARCOMA.** Irwin Feigin and Sidney W. Gross (by invitation), Pathology and Neurosurgical Departments of the Mount Sinai Hospital, and Pathology and Neurology Departments of the College of Physicians and Surgeons, Columbia University, New York, N.Y.

*Abstract.* In three instances tumors of the cerebrum have been studied which presented an admixture of two dissimilar tissues, both neoplastic. One tissue resembled the glioblastoma multiforme, being composed of small dark cells diffusely distributed in an eosinophilic, fibrillar matrix. These fibers resembled glial fibers in that they were stained blue with the phosphotungstic acid-hematoxylin method, and pink with the azocarmine technique. The other tissue resembled a fibrosarcoma, being composed of cells with elongated or fusiform, moderately chromatic nuclei arranged in parallel rows along with deeply eosinophilic, coarse fibers. These fibers resembled collagen in that they were stained blue by the azocarmine method and tan with phosphotungstic acid hematoxylin. Some of these fibers were argyrophilic by

the Wilder method. The neoplastic character of each of these tissues was manifested by the presence of mitotic figures, high cellularity, and significant atypism and variability. Portions of each tissue were necrotic.

In the glioblastomatous areas, the marked hyperplasia and hypertrophy of the cells of the vessel walls, characteristic of this tumor, were clearly evident. In areas the appearance suggested that neoplastic changes had occurred in some of these hyperplastic vessel walls, as indicated by increased cellularity and a significant atypism, including the presence of bizarre, deeply stained, elongated nuclei. The interpretation is offered that the neoplasm was originally glioblastomatous in character, and that the factor which commonly induces vascular hyperplasia induced, in these instances, a sarcomatous change in the mesenchymal cells of the vessel walls. There is evidence, particularly in one case, that the sarcomatous element may overgrow the glioblastomatous tissue, so that some cases considered primary sarcoma of the brain substance may have originated in this fashion.

**CHANGES IN THE BRAIN FOLLOWING SMALLPOX VACCINATION.** Vera B. Dolgopod and (by invitation) Morris Greenberg and Rosa Aronoff, Willard Parker Hospital, New York, N.Y., Department of Health, New York, N.Y., and Greenpoint Hospital, Brooklyn, N.Y.

*Abstract.* In the month of April, 1947, some 5 million persons received smallpox vaccination following the appearance of several cases of smallpox in New York City. Of 4 patients who died with neurologic manifestations suggestive of postvaccination encephalitis, 2 showed no pathologic changes in the brain and 2 presented microscopic findings in the central nervous system which differed from the picture encountered in postvaccination encephalitis. Brain lesions were encountered in 2 other cases in which encephalitis was not suspected. One patient was a child with generalized infantile eczema who developed eczema vaccinatum after exposure to vaccinated parents. The brain showed perivascular cuffing, but no damage to the myelin. The second child developed pertussis 1 week following vaccination. Severe paroxysms, pneumonia, muscular twitchings, and hyperthermia developed; death occurred 6 weeks after vaccination. Glial collections in the white matter bordering on several veins were encountered, producing areas of replacement of myelin. The picture was suggestive of residual encephalitis of postinfectious type, as seen after vaccination, and was different from the changes observed in pertussis. Pneumonia was the cause of death.

**ACUTE HEMORRHAGIC LEUKO-ENCEPHALITIS. ITS RELATIONSHIP TO THE DEMYELINATING DISEASES.** John Moossy, Abner Wolf, and David Cowen (all by invitation), College of Physicians and Surgeons, Columbia University, New York, N.Y.

*Abstract.* Acute hemorrhagic leuko-encephalitis is marked by perivascular foci of necrosis, edema, hemorrhage, and acute inflammation in the white matter of the brain. These may coalesce into more diffuse, larger lesions. This condition has been related to the demyelinating diseases on the one hand and its lesions compared to those of experimental "allergic" encephalomyelitis on the other. The 2 cases reported here bear upon these points. A 6½-year-old boy had progressive, diffuse sclerosis of the cerebrum dating from early infancy. Coupled with this were more acute hemorrhagic lesions in the brain stem. This co-existence in the same individual of a diffuse, chronic demyelinating process and a hemorrhagic leuko-encephalitis strengthens the belief that the latter is related to the demyelinating disease in its pathogenesis. The second case was that of a 6-year-old girl with asthma and other allergic phenomena, who developed hemorrhagic leuko-encephalitis of the right



cerebral hemisphere following prophylactic pertussis vaccine injections in the absence of pertussis. This suggests an immunologic mechanism in the pathogenesis of the lesions and forms a further link with experimental "allergic" encephalomyelitis which, it has been shown, bears a striking histologic resemblance to hemorrhagic leuko-encephalitis.

**IDIOPATHIC DEMYELINATING DISEASE IN YOUTH (SCHILDER'S DISEASE).** L. J. McCormack (by invitation), Cleveland Clinic, Cleveland, Ohio.

*Abstract.* The presentation concerns a rare form of disseminated demyelination of the central nervous system. The lesions were studied in 2 cases, the patients both adolescent boys, and demonstrated the characteristics of the acute and chronic phases of the disease. The case of short duration (7 weeks) showed what we believe to be one of the earliest stages that has been studied. The clinical features were those of an ascending myelitis with late development of nystagmus and diplopia. Death was due to respiratory complications. Areas of relatively acute demyelination were present generally, but were especially prominent and large in the occipital lobes. The configuration suggested the possibility of successive waves of demyelination. The second case, of at least 10 years' duration, demonstrated the changes found in the chronic phase of the disease. The prominent clinical features were retardation of growth and mental development, deafness, spasticity of the lower extremities, and ocular muscle palsies. Broad areas of myelin loss were present throughout the cerebral hemispheres and cerebellum.

A comparison of the lesions of the 2 cases revealed that those of the first were sharply margined, smaller in size, and contained many lipid-laden macrophages. In the second case the areas of demyelination were hazily outlined and without macrophage activity. In neither instance was there sclerosis or association with blood vessels. The clinical complaints could be correlated roughly with the anatomical findings.

**COMPRESSION OF THE SPINAL CORD BY EXTRAMEDULLARY NEOPLASMS. A CLINICAL AND PATHOLOGIC STUDY.** Howard J. McAlhany (by invitation) and Martin G. Netsky (by invitation), John Gaston Hospital, Memphis, Tenn., and Montefiore Hospital, New York, N.Y.

*Abstract.* This report is based on a study of 19 cases of extramedullary spinal cord tumors. It is shown that extramedullary tumors produce a variety of symptom complexes which are not related to their location in the horizontal plane of the spinal cord. Pain is the usual initial symptom of extramedullary compression and paraplegia is the usual outcome. The intervening symptoms do not appear in a definite pattern. The anterior columns are less vulnerable to pressure regardless of the location of the tumor in the horizontal plane. Reasons are presented for doubting the importance of the dentate ligaments in the pathogenesis of spinal cord compression. Coup and contrecoup effects are most commonly produced by extramedullary masses. The mechanism of spinal cord compression is believed to be primarily mechanical and secondarily related to the collapse of small intramedullary blood vessels.

**HEREDOPATHIA ATACTICA POLYNEURITIFORMIS: THE NEUROPATHOLOGIC CHANGES IN THREE ADULTS AND ONE CHILD.** Jan Cammermeyer (by invitation), Webb Haymaker, and Sigvald Refsum (by invitation), Armed Forces Institute of Pathology, Washington, D.C., and Department of Neurology, University of Bergen, Bergen, Norway.

*Abstract.* The cases are from three affected families. Case 1 (family B of Ref-

sum), 28-year-old male. This is the only case in which peripheral nerves and muscles were available. The nerves showed characteristic hypertrophic interstitial polyneuritis and some muscles were atrophic. No central nervous system lesions were found. Cases 2 and 3 (family A of Refsum). Case 2, 41-year-old male. The changes consisted of focal degeneration of the decussation of the brachium conjunctivum, lemnisci, pontocerebellar fibers, pontile pyramidal fibers, and white matter adjacent to the dentate nucleus. Case 3, 32-year-old female, sister of the patient known as case 2. A few spinal rootlets contained scattered fat-filled macrophages. The nuclei pontis showed degeneration and there was periventricular gliosis. Case 4 (family of Refsum, Salomonsen, and Skatvedt), a 9-year-old girl. Nerve cells of the inferior olivary nucleus were decreased and there was Marchi degeneration and gliosis of the olivocerebellar tracts.

In all 3 adult cases there was an extraordinary collection of neutral fat in the leptomeninges, perivascular adventitial cells, epithelial cells of the choroid plexus, and interstitially in the pallidum; it was also seen in scattered nerve cells elsewhere in the central nervous system. In the fourth case, fat studies on the central nervous system were inconclusive. As to the viscera, conspicuous amounts of fat were found in the liver and kidneys in case 1. Fat studies were not performed on the other cases. The nerve changes in case 1 and in a case of Refsum's syndrome described by Reese and Bareta (1950) indicate that in some cases, at least, the disorder is closely related to Dejerine-Sottas disease. The degenerative changes in the central nervous system in our cases were focal and diverse. The lack of a pattern of degeneration distinguishes the disorder from other hereditary diseases of the central nervous system.

**STUDIES ON LIPIDOSIS OF THE CENTRAL NERVOUS SYSTEM.** Stanley M. Aronson (by invitation) and Bruno W. Volk, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, N.Y.

**Abstract.** A clinical and anatomical study of 9 patients with central nervous system lipidoses is presented. The series is comprised of cases of Tay-Sachs' disease, Niemann-Pick's disease, and gargoylism. In so far as the central nervous system is concerned, there are no indisputable morphologic distinctions among the three diseases. The basic histologic changes reside in the neurons and consist of cytoplasmic distention with a faintly sudanophilic refringent material. The Nissl substance is decreased and the residuum compressed about an eccentric nucleus. In spite of profound changes within such affected cells, the neurofibrils persist until neuronal dissolution. No cytologic or cytochemical individuality can be established among the affected neurons of the various lipidoses. In all instances the abnormal process within ganglion cells is apparent throughout the central nervous system as well as in more remote neurons (*viz.*, autonomic nervous system, retina, and posterior lobe of the pituitary body).

Following neuronal breakdown, the adventitious lipid is absorbed by reactive microglial elements. These phagocytic cells migrate toward neighboring perivascular spaces and ultimately appear within the leptomeninges. The presence of abnormal lipid in the subarachnoid space evokes a fibroblastic reaction which is reflected grossly as a diffuse meningeal thickening and opacity. In cases surviving for a longer period, the degree of fibrosis becomes marked, hindering absorption of cerebrospinal fluid and eventuating in a communicating hydrocephalus. Pneumoencephalograms of 5 such cases showed perceptible ventricular dilatation in all instances. Spinal fluid from all cases revealed a moderate increase in protein and, except for the case of gargoylism, an abnormally diminished gamma globulin fraction. Many of the lipid-filled macrophages which distend the cerebral perivascular spaces degenerate before reaching the subarachnoid area. Endothelial cells within the cerebral parenchyma secondarily participate in scavenger activity and become laden with lipid. In 2 cases fibrin was deposited upon distended endothelial elements

with the development of multiple occlusive thrombi. Grossly there were numerous foci of typical encephalomalacia.

While the salient clinical features (amaurosis, arrested development, paralysis) arise from the diffuse neuronal dystrophy, a number of pathologic changes result from the secondary somatic reaction to the abnormal lipids released following breakdown of affected neurons (meningeal fibrosis, hydrocephalus, encephalomalacia).

**HISTOCHEMICAL STUDIES ON THE CEREBRORETINAL DEGENERATIONS AND OTHER LIPID METABOLIC DISORDERS.** Benjamin H. Landing and David G. Freiman, Children's Hospital Research Foundation and Department of Pathology, Cincinnati General Hospital, Cincinnati, Ohio.

*Abstract.* The results of comparative histochemical studies of the abnormal ganglion cells in patients with different forms of cerebroretinal degeneration, including Tay-Sachs' infantile form, Bielschowsky's early juvenile form, and Spielmeyer-Vogt's late juvenile form, are presented. Histochemical features common to the members of this group and to the similar ganglion cell changes in Niemann-Pick's disease are discussed, as well as histochemical differences among the various conditions. Differences among the ganglion cells in these conditions and the enlarged ganglion cells of infantile muscular atrophy (Werdnig-Hoffmann's disease) are presented, and a histochemical comparison of such ganglion cell lesions and the visceral lesions of Niemann-Pick's, Gaucher's and Letterer-Siwe's disease is made. Evidence from these studies bearing on "amyloid" degeneration of ganglion cells in familial myoclonus epilepsy is also discussed.

**HYDRANENCEPHALY.** James B. Arey and Henry W. Baird, III (by invitation), St. Christopher's Hospital for Children and the Departments of Pediatrics and Pathology, Temple University School of Medicine, Philadelphia, Pa.

*Abstract.* The clinical and pathologic findings in 2 infants with hydranencephaly are reported. In one infant the "ventricular" cavity was infected (pyo-anencephaly) with resultant secondary changes which obscured the remaining structures. In the second infant, in whom the diagnosis of cytomegalic inclusion disease had been established during life, the structures were more readily visualized. The cerebral hemispheres were replaced by a huge unilocular cavity, in the floor of which were the thalami and remnants of the cerebral cortex. Supero-laterally the walls of the single "ventricle" consisted of a thin, delicate membrane. The mesencephalon, pons, medulla, and cerebellum were clearly visualized.

The diagnosis of hydranencephaly can be established during life by pneumo-encephalography, transillumination of the head, electro-encephalography, or craniotomy. The clinical course of the condition, and often the contour of the head, tend to differentiate hydranencephaly from hydrocephalus. At necropsy they can be differentiated by the presence of a recognizable cerebral cortex in the latter, whereas this is in large part replaced by a delicate membrane in the hydranencephalic infant. Islands of cortical tissue may, however, remain and be responsible for some electrical activity demonstrable by electro-encephalography. Particular emphasis is placed upon obstruction of the aqueduct of Sylvius, which was noted in both of these infants. This change, the significance of which has not been recognized previously, is discussed in relation to the various theories of pathogenesis.

**MYELOMALACIA AND MULTIPLE CAVITATIONS OF THE SPINAL CORD SECONDARY TO ADHESIVE ARACHNOIDITIS: AN EXPERIMENTAL STUDY.** Orville T. Bailey and (by invitation) Robert L. McLaurin, Peter H. Schurr, and Franc D. Ingraham, Larue D. Carter Memorial Hospital, Indianapolis, Ind., Christian R. Holmes Hospital, Cincinnati, Ohio, Maudsley Hospital, London, England, and Children's Medical Center, Boston, Mass.

*Abstract.* Chronic adhesive arachnoiditis was produced in 45 dogs by the intra-

cisternal injection of pantopaque or kaolin. Twenty-five of the dogs developed myelomalacia with multiple cavitations in the cervicothoracic portions of the spinal cord. The necrosis and cavitations were judged to be due to ischemia resulting from vascular compression in the meninges by the fibrosing arachnoiditis. The lesion began at the junction of gray and white matter in the posterior portion of the spinal cord. As it extended, it involved the posterior area of the spinal ependyma, thus allowing communication between the cavitations and the cerebrospinal fluid pathway. The localization of the process could be explained by the fact that the lower cervical region is especially susceptible to ischemia because of the transition of arterial supply in this area from vertebral arteries to segmental radicular vessels. Communicating hydrocephalus also was produced in nearly all animals of the series. That pressure downward at the foramen magnum was not an important factor producing the cavitations was shown by the extension of the process of necrosis and cavitation well down into the thoracic region, the lack of correlation between the degree of hydrocephalus and the severity of spinal cord damage, and the irregular distribution of the early changes in the spinal cord.

The experimental lesions bore a considerable resemblance to certain instances of human syringomyelia and to the consequences of occlusion of spinal arteries in clinical patients.

**THE ULTRASTRUCTURE OF NERVE MYELIN AND ASSOCIATED STRUCTURES.** Francis O. Schmitt (by invitation), Biology Department, Massachusetts Institute of Technology, Cambridge, Mass.

*Abstract.* This report deals primarily with the application of physical methods (chiefly electron microscopy, x-ray diffraction, and polarization optics) in the investigation of the molecular structure of nerve myelin. In addition, consideration is given to the structure of the lipid-protein complexes which are homologous to the myelin sheath in various invertebrates. In order to interpret the layered structure of such lipids and lipid protein complexes as seen in electron micrographs of very thin sections, a program of investigation has been initiated, in collaboration with Dr. Betty Ben Geren (Children's Cancer Research Foundation, Boston), on samples of pure lipids and lipid-protein complexes. The interlayer period is observed in sections of fixed material and compared with that deduced from x-ray diffraction patterns from the same samples. Finally the structure of the Schwann cell is discussed, with particular relation to the laying down and maintenance of the organized structure of the myelin.

**THE NATURE OF GLIOMAS AS REVEALED BY ANIMAL EXPERIMENTATION.** H. M. Zimmerman, Laboratory Division, Montefiore Hospital, New York, N.Y.

*Abstract.* Human brain tumors of the glioma variety encompass at least eight universally recognized, distinctive types as well as a number of related subtypes. Almost every glioma contains, in addition to the distinctive cells from which its name is derived, variable numbers of other glial elements. Classification of a gliogenous neoplasm is thus based upon the identification of the most malignant cells present and/or the predominance of one kind of cell. This method of classifying gliomas is justified by clinical experience and a knowledge of their biologic characteristics.

It is generally accepted that the different glial cells of the mature nervous system, with the possible single exception of the microglia, are all derived from embryonal neuroectoderm. This origin the glia share in common with the neurocytes. Further, the primitive neuroectoderm (medullary epithelium) differentiates by recognizable stages to the fully mature glia. These adult cells may, according to one popular concept, give rise under certain conditions to glial neoplasms by dedifferentiation.

Implicit in this concept are two conditions: (1) that a single adult glial cell undergoes malignant change and by proliferation gives rise to the neoplasm; (2) that the proliferating cells proceed through stages of dedifferentiation ultimately to achieve degrees of uniformity which permit classification.

Animal experimentation with gliomas produced with chemical carcinogens has contributed to the clarification of a number of the problems which surround this type of human neoplasm. It has been shown that many different adult and morphologically distinct glial cells begin to proliferate under chemical stimulation almost simultaneously to produce a neoplasm. The resulting glioma is therefore almost never a "pure" tumor, *i.e.*, composed of one cell type, but rather a mixture of several different cell types. In a true sense, therefore, nearly every glioma is multipotential, by which is meant that it has the ability to become one or another of the eight major types of this class of tumor. Transplantation experiments have actually proved the validity of this concept, for by judicious selection of explants from a mixed glioma, multiple "pure" gliomas may be created by transplantation into homologous animal species. This experience justifies the view that all gliomas are really variants of one tumor type, in the sense that Hodgkin's granuloma, lymphosarcoma, and reticulum cell sarcoma are variants of malignant lymphoma.

Experimentation with gliomas has expanded knowledge of their histogenesis along two fronts. It has demonstrated that certain gliomas have a predilection for certain regions of the brain: ependymomas occur when carcinogenic agents are placed in contact with the ventricular wall; medulloblastomas originate almost exclusively in the cerebellum; oligodendrogliomas arise in the subcortical white matter of the cerebral hemispheres. It has also demonstrated that environmental factors are important in determining tumor type. An example of this environmental influence is the mouse ependymoma which grows as an undifferentiated malignant glioma in the chick embryo and reverts again to an ependymoma on transplantation to the mouse.

**A SURVEY OF SOME RECENTLY DEVELOPED HISTOCHEMICAL METHODS FOR ENZYMES OF NERVOUS TISSUE.** Arnold M. Seligman (by invitation), Yamins Laboratory for Surgical Research, Beth Israel Hospital, Boston, and Department of Surgery, Harvard Medical School, Boston, Mass.

*Abstract.* Although methods for a variety of enzymes have been developed which can be applied to sections of nervous tissue prepared from paraffin embedded blocks, available methods for some of the enzymes will not tolerate fixation and embedding in paraffin. It was therefore important to develop methods for frozen sections of nervous tissue. Some of the older methods, particularly methods dependent upon production of azo dyes, were unsatisfactory because of the lipid solubility of the dyes and their diffusion into myelin. New methods have been developed which result in the production of dyes less soluble in myelin, such as indigo or azo dyes containing an amide linkage. Methods are now available for acid and alkaline phosphatase, esterase, serum cholinesterase, acetylcholinesterase,  $\beta$ -D-glucuronidase, and succinic dehydrogenase. Formulation of these methods is given, and the results with some are shown in normal nervous tissue.

**QUANTITATIVE HISTOCHEMICAL ARCHITECTONIC PATTERNS IN THE MONKEY CERE-BRAL CORTEX.** Eli Robins (by invitation) and David E. Smith, Departments of Neuropsychiatry and Pathology, Washington University Medical School, St. Louis, Mo.

*Abstract.* To meaningfully correlate biochemical patterns with histologic structure in an architecturally complex organ such as the brain requires the use of quantitative chemical methods of sufficient sensitivity that they can be applied to



samples of tissue small enough to contain at most only a few histologic elements or, ideally, to a single cell or its constituents. The quantitative histochemical method used in this study consists of the isolation by manual microdissection of samples weighing from 1 to 5  $\mu\text{g}$ . of dry weight from selected regions in sections of frozen-dried, unembedded tissue. The dissected samples are weighed on a Lowry quartz-fiber balance, placed in a special small test tube, and analyzed by quantitative microtechniques utilizing the standard principles of macrovolumetric quantitative analysis. Quantitative determinations of protein, cholesterol, and various enzymes were made in each of the cytoarchitectonic layers and their major subdivisions as well as the subjacent white matter in the motor and visual cortices of the monkey. The enzymes determined included acid phosphatase, alkaline phosphatase, aldolase, fumarase, adenosinetriphosphatase, malic dehydrogenase, lactic dehydrogenase, purine nucleoside phosphorylase, and glutamic dehydrogenase. Enzymatic activities, in general, showed an ascending gradient from the values in white matter to those in the most superficial cortex. These activities increased in a more regular manner through the layers of the motor cortex than through those of the visual cortex. In the motor cortex the gradient varied from five-fold for malic dehydrogenase to less than two-fold for purine nucleoside phosphorylase.

**SOME ASPECTS OF THE STRUCTURE OF MYOSIN.** Andrew G. Szent-Gyorgi (by invitation), Institute for Muscle Research, Woods Hole, Mass.

**Abstract.** Short trypsin digestion of myosin decreases its viscosity but leaves its ATPase activity unaltered. In this reaction myosin is split into two components. The component with the higher molecular weight, heavy-meromyosin, has all the ATPase activity and actin-combining capacity of the intact myosin molecule. The component with the smaller molecular weight, light-meromyosin, is an asymmetric molecule and has a tendency to form structures such as are shown by electron microscopic studies. There are four light-meromyosins and two heavy-meromyosins in a single myosin molecule. The possibility that it is the light-meromyosin which folds in the shortening of myosin is examined. The light-meromyosin is built of further sub-units, called protomyosins. This component can be obtained by treating the light-meromyosin with urea under well defined conditions. The protomyosin appears to be homogeneous as far as size and shape are concerned, with a molecular weight of about 3,500, but is heterogenous in the electrophoretic experiments. The 25 to 30 protomyosins make a light-meromyosin molecule, mainly by end to end association. In the depolymerization of light-meromyosin into protomyosin there is no appearance of new carboxylic groups, indicating that the protomyosins are not connected *via* peptide links. The possibility of the protomyosins being the final contractile units and the implications of the structure of myosin in the contractile process are discussed.

**THE STRUCTURAL AND METABOLIC RELATIONSHIP BETWEEN CYTOCHONDRIA AND MYOFIBRILS STUDIED BY PHASE MICROSCOPY, ELECTRON MICROGRAPHY, AND MICROKINEMATOGRAPHY.** John W. Harman, Department of Pathology, University of Wisconsin Medical School, Madison, Wis.

**Abstract.** Conventional light microscopy reveals the interstitial granules between myofibrils of skeletal muscle. By homogenization of muscle in isosmotic non-electrolyte media and differential centrifugation, pure suspensions of myofibrils and mitochondria are obtained. Cytochondria are further identified by phase microscopy and separated into rodlet mitochondria and sarcosomes. Neither myofibrils nor sarcosomes possess significant oxidative capacity, which is confined to the mitochondria where the integrated system of enzymes implementing the Krebs cycle is

located. Mitochondria have a  $Q_{O_2}$  (N) of 1080 and oxidatively phosphorylate with P/O ratios up to 3 during oxidation of glutamate and  $\alpha$ -ketoglutarate. The oxidative phosphorylation is selectively protected by added serum albumin with stabilization of consistently high P/O ratios. The sarcosomes significantly augment the rate of mitochondrial oxidation. Differential counts by phase microscopy establish the related latent rodlets, active target forms, and degenerate crescents in association with metabolic activity. Concomitant studies of total homogenates correlate the contractility of myofibrils with the average mitochondrial state; as mitochondrial degeneration proceeds and oxidative phosphorylation fails, the myofibrils cease to manifest rhythmic reversible contractions. Cinematographic studies by phase microscopy of myofibrillar contractions demonstrate three varieties: (1) a reversible normal contraction in which the relative movements between and in A and I bands are observed; (2) a conversion inversely by slow contraction to 60 per cent of the original length corresponding to Ramsey's "delta" state; (3) the syneresis of exhausted or "delta" fibrils to 10 per cent of the resting length by addition of excess ATP and magnesium. The latter two types are observed after failure of the mitochondrial metabolic system or glycolytic system which sustains rhythmic contractions. Since myofibrils contain no demonstrable oxidative or glycolytic enzymes, they depend on the mitochondria for  $\sim$ P energy supply, so agents which injure mitochondrial metabolism, e.g., dinitrophenol, accelerate myofibrillar degeneration. Electron micrographs show that mitochondria are united directly to the myofibrils by fine reticular strands which maintain close approximation to A bands; the bands may have functions as conduits as well as bands. The mitochondria have no discernible membranes; some appear homogeneous and others show regular lamellated structure, suggesting a spiral arrangement of the gel, although cristae are not evident. They are attached to adjacent sarcosomes by fine strands.

**ELECTRON MICROSCOPIC STUDIES OF MUSCLE.** David Spiro (by invitation), Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass.

*Abstract.* This paper reviews briefly electron microscopic investigations on the structure of striated muscle carried out during the past few years at the Biology Department, Massachusetts Institute of Technology. The major contributions consist of studies done by Huxley and Hanson, as well as work by Hall, Jakus, Schmitt, Hodge, and Spiro. The early work in which fragmented muscle was used disclosed the myofibrils to be composed of regularly arrayed parallel filaments which had an axial periodicity of 400 Å. The development of suitable techniques for obtaining sections thin enough for examination in the electron microscope enabled one to study the transverse structure of the myofibril and the latter was found to be composed of filaments packed in a hexagonal array. Further studies by Huxley led to the observation that there are two types of filaments within the myofibril. Finally, Hanson and Huxley were able to demonstrate that one of the types of filaments represented the protein actin and the other type was composed of myosin. Typical electron micrographs were shown.

**INTRAMEDULLARY LIPOMA OF CERVICAL SPINAL CORD.** George Margolis and William M. Berton (by invitation), Department of Pathology, Duke University School of Medicine, Durham, N.C.

*Abstract.* A rare example of diffuse involvement of the cervical spinal cord by an intramedullary lipoma is reported. The complete effacement of the cord and the intimate relationship of the adipose tissue and the neural structures constituted the striking features of the lesion. The genesis of the tumor, its relationship to the skeletal deformity, and the anatomical and functional effects of the lesion are discussed.



**CORPUS CALLOSUM LESIONS FOLLOWING BLUNT MECHANICAL TRAUMA TO THE HEAD.** Richard Lindenberg (by invitation), Russell S. Fisher, Stanley H. Durlacher, and (by invitation), William V. Lovitt, Jr., and Ella Freytag, Division of Legal Medicine, University of Maryland Medical School and the Central Anatomic Laboratory of the Maryland State Department of Mental Hygiene, Baltimore, Md.

*Abstract.* Traumatic lesions in the corpus callosum, rarely described in the literature, are a frequent result of mechanical trauma to the head. We have found such injuries in 51 cases or 16 per cent of all head injuries due to blunt mechanical trauma examined at the Office of the Chief Medical Examiner of Maryland in the last 2 years. The gross alterations consist of hemorrhages, ischemic necrosis, or hemorrhagic softenings. In 9 cases the lesions extended over the entire length of the corpus callosum; in 21, from one third to two thirds of the corpus was involved, while in the other 21 the lesions were of small focal character. The survival time ranged from a few minutes to 8 months. There was no relationship between the absence or occurrence of skull fracture and the corpus callosum damage. The lesions were frequently accompanied by other traumatic foci in neighboring structures such as the septum pellucidum, fornices, caudate nuclei, dorsal thalami, and gyri cinguli. In 48 cases the impact area was located above the level of the corpus callosum; in 3 instances near the foramen magnum. The direction of the force was uniformly from vertex toward the base of the skull, or vice versa. As in cortical contusions, the lesions originate at the moment of impact. They are caused by two principal factors. One is bilateral stretching of the corpus callosum due to elastic flattening of the skull with elongation of the transverse diameter at the moment of impact. The second is pressure and shearing effect due to localized displacement of brain tissue in the path of the transmitted force. In most cases both mechanisms are operative. Contrary to expectation, cutting by the sharp edge of the falx is a rare occurrence. The similarity of these injuries to other lesions of hitherto unrecognized etiology, especially agenesis of the corpus callosum, is stressed. The principal significance of the findings of this study, however, is in the insight gained into the events occurring within the cranium at the moment of blunt impact.

**POST-TRAUMATIC CIRCULATORY LESIONS OF THE BRAIN.** Karl T. Neubuerger, University of Colorado School of Medicine and General Rose Memorial Hospital, Denver, Colo.

*Abstract.* Traumatic injuries with only minor involvement of the head, often without fractures of the skull, and usually without gross mechanical laceration and destruction of its contents may produce the following: areas of anemic softening in and around the basal ganglia and multifocal cortical necrosis within hours to days; hemorrhagic cortical infarcts, plasmatic vascular exudate, and varying degrees of edema within days to weeks; disseminated cortical degeneration and small hemorrhages in leptomeninges and cortex within weeks to months; and massive intracerebral hemorrhages, the so-called delayed traumatic apoplexies, within days to months. Pre-existing vascular lesions or coarse traumatic destructions of vessels do not play a major rôle in these events. The pathogenesis of both early and late changes is probably similar. Factors such as concussion, shock, prolonged local interference with vasomotor function, vasoparalysis, vasothrombosis, disturbances in the barrier function of the vascular endothelium, and possible damage to centers in the medulla oblongata must play a rôle. Increased intracranial pressure with herniation of the brain stem and blockage of venous return may be operative in delayed traumatic apoplexy of the pons. The significance of post-traumatic angioneurosis should not be over-rated. Pertinent cases were demonstrated and the similarity of certain features to those of anoxia were discussed. Concurrent changes in other organs having similar pathogenesis were mentioned.

**CRYPTOCOCCOSIS OF THE CENTRAL NERVOUS SYSTEM IN DOMESTIC ANIMALS.** John T. McGrath (by invitation), University of Pennsylvania Veterinary School, Philadelphia, Pa.

*Abstract.* Cryptococcosis invasion of the central nervous system is a relatively uncommon occurrence in both man and animals, if its incidence is based on reported cases. A survey of literature, both medical and veterinary, discloses that a comparatively small number of cases have been reported since the disease was first described. Weidman and Ratcliffe have reported invasion of the central nervous system in a cheetah with generalized torulosis (cryptococcosis) from the Philadelphia Zoological Gardens. During the past 5 years 10 cases of blastomycosis have been necropsied at the Veterinary School, University of Pennsylvania. In 6 cases (3 cats, 2 dogs, 1 horse) the animals clinically showed neurologic disturbances with subsequent necropsy findings indicative of cryptococcosis (torulosis, European blastomycosis). At necropsy, 3 cases presented typical lesions consisting of milky, thickened leptomeninges with small cyst-like cavities throughout the substance of the brain. Freshly stained smears revealed typical budding yeast bodies. In 2 cases gross lesions of meningitis were noted, the organisms being observed histologically and obtained by culture. In the horse the lesions, for the most part, were histologic, consisting of focal chronic meningitis with lymphocytes and mononuclear and giant cells. Typical organisms were observed, both intracellular and lying free in the meninges.

**EVIDENCE FOR AN INFLAMMATORY FACTOR IN THE PATHOGENESIS OF CEREBROVASCULAR ANEURYSMS.** Fred P. Handler (by invitation) and Herman T. Blumenthal, Department of Pathology, the Jewish Hospital, St. Louis, Mo.

*Abstract.* Two distinct types of aneurysms were encountered in the course of a study of aging changes in the basilar artery. The more frequent form was on an arteriosclerotic basis, but the second type, which appeared to be inflammatory in nature, was seen in approximately 6 of 150 cases studied. At focal points along the involved vessel, clusters of polymorphonuclear leukocytes infiltrated from the vasa vasorum into the wall. This was followed by granulation tissue with disruption of the media and internal elastic lamella, while the character of the cellular reaction changed to small mononuclear cells. In one instance herniation of the internal elastic lamella through the medial defect produced a distinct bulge on the adventitial surface. Such an event need not terminate in rupture, but rather in organization. In healed examples, fibrous tissue occupied a superficial crescentic position in the media adjacent to the disrupted and often broken internal elastic lamella. In one instance an aneurysm of the anterior communicating artery was surgically isolated, but the patient subsequently expired. Well localized inflammatory lesions were found at some distance from the definitive aneurysm. The cellular reaction and the sharp demarcation were characteristic of the lesions described above.

The etiology of cerebrovascular aneurysms is discussed in relation to other vascular lesions of inflammatory type without specific etiology. In this series no instance of aneurysm on the basis of a congenital muscular defect was observed.

**ORGANIC BRAIN DISEASE IN THE AGED.** Abe Towbin (by invitation), Department of Pathology, Ohio State University Medical School, Columbus, Ohio.

*Abstract.* In a study of the central nervous system in 505 consecutive necropsies, 341 brains showed significant organic changes. The cases studied were from an institutional population composed largely of indigent subjects of the older age group. Changes observed at necropsy were correlated with the mental status of the patients prior to death.

The preponderance of chronic organic brain disease proved to be due to four processes: cerebral arterial sclerosis; senile encephalopathy; cerebral syphilis; cere-

bral embolism. In 26 per cent of the cases with chronic organic brain disease, the primal lesion was sclerosis (atherosclerosis) of main cerebral arteries with associated medium to large infarcts. Clinically this was associated with a relatively sharp onset of motor, mental, or other circumscribed symptoms; the average age at death was 69 years. In 27 per cent of cases senile encephalopathy was the prominent pathologic feature. The brains were small and showed convolucional atrophy and *ex vacuo* hydrocephalus. Microscopically, sclerosis of small intrinsic vessels was associated with perivascular encephalomalacia. The average age at death was 75 years. In 24 per cent of the cases the brains showed the combined effects of arterial sclerosis and senile encephalopathy. Paresis accounted for 12 per cent of chronic brain disease in this series. Chronic subdural hemorrhage and other post-traumatic lesions comprised 3 per cent; neoplasms were present in 3 per cent.

Cerebral embolism was shown at necropsy to be responsible for 5 per cent of chronic organic brain disease. The group having cerebral embolism was composed of relatively younger persons; myocardial infarction and intracardiac mural thrombi, clinically silent and antedating the cerebral disease, were commonly present at necropsy. The brain generally presented scattered small and medium-sized infarcts of varying age and an absence of arterial sclerosis. The embolic nature of the disease was rarely recognized before death; the clinical pattern generally resembled that of arteriosclerosis, and the cases were generally so diagnosed.

#### HISTOPATHOLOGIC PATTERNS OF SELECTIVE BRAIN DAMAGE FROM VARIOUS CAUSES.

Kornel L. Terplan and (by invitation) Sarah Barnes, Buffalo General Hospital, Buffalo, N.Y.

**Abstract.** In order to study the selective sensitivity of the various areas of the central nervous system, including brain stem and pallium, a fairly complete gross and histologic analysis is mandatory. The diffuse destruction observed in certain cases of acute virus encephalitis, including the herpes group, particularly in the frontal and temporal lobes, is contrasted with the entirely selective and comparatively minor damage to the motor cortex in poliomyelitis and with the different pattern of subependymal damage in the infant in so-called inclusion body disease. The changes observed from anoxia, as particularly in delayed death from anesthesia, demonstrate, on the other hand, the pronounced involvement of the occipital lobe and of the striatum, both of which are usually much less affected, or not at all, in virus encephalitis. It is shown that the histologic picture in so-called demyelinating processes, like measles encephalitis, can be considerably altered by anoxic factors. Finally, the changes occurring in porphyria are presented, which show again a peculiar selection of certain areas in the white matter with segmental inclusion of the cortex.

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#### STUDY OF THE ELIMINATION OF RADIOACTIVE DIODRAST FOLLOWING A SINGLE RAPID INTRAVENOUS INJECTION IN DOGS. Rafael Dominguez and (by invitation) William C. Schmidt, Division of Pathology, St. Luke's Hospital, Cleveland, Ohio.

**Abstract.** The blood concentration of radioactive diodrast was recorded continuously from the beginning of the injection until it fell near the background radioactivity level. In other experiments the blood diodrast concentration was determined in venous blood samples obtained at selected intervals. In all but three of the experiments carrier was added. Renal and extrarenal clearances were determined. Extrarenal clearances were also calculated in dogs without renal function. Bile clearances were directly determined in some of the experiments. Other determinations include hematocrit, ratio of plasma to whole blood activity and assay for radioactivity of selected organs and tissues post mortem. After an initial period, varying between  $\frac{1}{2}$  and 2 hours, the diodrast concentration in the blood falls ex-

ponentially with the time. In some of the experiments the first phase of the blood diodrast concentration has been reduced to a diffusion phase either by continuous intravenous reinjection of the urine for about 1½ hours or by nephrectomy. A few of the blood curves were shown. Several methods of computing the volume of distribution were discussed.

#### EXPERIMENTAL PRODUCTION OF PIGMENTED VILLONODULAR SYNOVITIS IN DOGS.\*

J. M. Young (by invitation) and A. G. Hudacek (by invitation), Laboratory Service, Veterans Administration Medical Teaching Group, Kennedy Veterans Administration Hospital, Memphis, Tenn.

**Abstract.** This condition has been termed xanthoma, xanthogranuloma, villous arthritis, hemorrhagic villous synovitis, myeloplaxoma, benign and malignant giant cell tumor of joints, and, more lately, villonodular synovitis. The pathogenesis of this lesion is not fully understood. A history of trauma, often not severe, frequently precedes the onset of villonodular synovitis, and the lesion usually requires months or years to develop fully. The patients often have periods of remission with rest. Weight-bearing joints are most often affected, particularly the knee.

Experiments were performed to study the changes produced by repeated hemorrhages into joints. The knee joints of dogs were injected repeatedly with blood obtained from the animals' jugular veins. This blood was not citrated or defibrinated. The experiment covered approximately 1 year. At first villous hyperplasia occurred, and this was most marked in the recessed portions of the joints, as stressed by Key in 1929. Later there was fusion of villi to form nodules and a pannus. Nodules form by two methods. The first is by fusion of villi. Blood often clots in the joints, and organization of these clots can form nodules of the second type, most often seen in the quadriceps pouch. The degree of pigmentation depends on the frequency of injections and the date of the last injection. Frequent injections produced deep pigmentation, but much of this disappeared if the joints were not injected for a month or more.

We believe that repeated joint hemorrhages are necessary for the development of pigmented villonodular synovitis. Trauma is often the initiating factor. Crushing of villi with continued use of the joint leads to further hemorrhage, and a self-perpetuating process results as long as the joint is used. When the fully developed lesion is present, rest does not result in complete resolution because of the advanced degree of nodule formation, the thick joint capsule, and extreme villous proliferation in the recesses of the joint.

#### EARLY CHANGES IN THE MOUSE KIDNEY AFTER EXPERIMENTAL BURN SHOCK. II.

TREATMENT WITH MOUSE PLASMA, PLASMA SUBSTITUTES (DEXTRAN, POLY-VINYLPYRROLIDONE, AND OXYPOLYGLUTAMIN) AND WHOLE MOUSE BLOOD. Robert W. Mowry and (by invitation) R. Carl Millican, University of Alabama Medical Center, Birmingham, Ala., and the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

**Abstract.** Hemoglobinuric nephrosis was found in the majority of mice subjected to a standardized procedure for producing experimental burn shock with 90 to 100 per cent mortality in 24 hours. Kidney changes during the first 24 hours include; (1) hemoglobinuria with hyaline droplets in the proximal tubular cells, presumably indicative of reabsorption, for about the first 6 hours; (2) numerous hemoglobin casts, chiefly in the distal convoluted and collecting tubules; (3) varying degrees of patchy, rarely extensive necrosis of the epithelial cells in Henle's loops, not usually seen before 4 to 6 hours after injury. Intravenous injection of 1 ml. of 0.9 per cent NaCl 1 hour after injury reduces the mortality to about 50 per cent, but failed to significantly modify the described histologic features.

\* This article will appear in a subsequent issue of *The American Journal of Pathology*.

Large numbers of mice were subjected to standardized burn injury. By rotation, individual mice were either not treated or were given an intravenous injection of 1 ml. of one of the following: mouse plasma, whole mouse blood, 6 per cent clinical dextran, 3.5 per cent polyvinylpyrrolidone (PVP), 5 per cent oxypolygelatin (OPG), or 0.9 per cent NaCl. Solutions of plasma substitutes were prepared in 0.9 per cent NaCl. Blood, plasma, and plasma substitutes were always given about 1 hour after the burn and simultaneously with both saline-treated and untreated controls. Three untreated and 3 saline-treated mice were killed hourly from 2 to 7 hours after injury, 6 from each group at 12 hours, and about 4 each at 24 hours after injury. One mouse was killed each hour from 2 to 7 hours with each of the other kinds of fluid therapy. Two mice from each of the other groups were killed at about 12 and 24 hours after the burn. Kidneys were examined by methods described in the earlier study. Features in the saline and the untreated groups were as expected. Treatment with any one of the plasma substitutes was associated with a substantial reduction in the number of hemoglobin casts. In addition, during the first 6 hours or so, the kidneys of plasma substitute-treated mice showed fairly extensive dilatation of nearly all levels of the nephron ("internal excretory hydronephrosis"). In the kidneys of mice given either dextran or gelatin the tubules contained copious amounts of the injected material. PVP was not demonstrated by us but is presumed to be present similarly in the renal tubules at this time. The degree of hemoglobinuria in the blood-treated mice appeared more advanced and hemoglobin cast formation unreduced. The kidneys of plasma-treated mice showed fewer hemoglobin casts and none of the features believed related to the renal excretion of the various plasma substitutes. The kidneys of plasma-treated mice appeared the most nearly normal. In spite of the reduction in hemoglobin casts, necrosis of the renal epithelium was still seen in Henle's loops, and was sometimes severe.

VIREMIA IN HAMSTERS INOCULATED WITH EQUINE ABORTION VIRUS.\* Charles C. Randall and (by invitation) W. C. Stevens and E. C. Bracken, Departments of Pathology and Biology, Vanderbilt University Medical School, Nashville, Tenn.

**Abstract.** Goodpasture first demonstrated that suckling hamsters inoculated with equine abortion virus developed liver lesions with intranuclear inclusions. One of us (C. C. R.) has demonstrated inclusions in hamster tissue maintained *in vitro*, but efforts to confirm the findings in the intact animal met with failure until Doll (1953), after years of painstaking and careful effort, succeeded in adapting the virus to the hamster, resulting in a severe hepatitis with intranuclear inclusions. In the present study an attempt was made to adapt liver passage virus (Doll) to the brain of 3 to 4 weeks-old hamsters. Serial intracerebral passage (10 to date) of hamster brain regularly produced hepatitis and death in 36 to 48 hours with abundant intranuclear inclusions. Brain suspension from a late passage regularly killed young animals at a dilution of  $10^{-3}$ . Numerous hematoxylin and eosin-stained sections of brain from various passages failed to show any inclusions, in contrast to the marked hepatitis in all animals. It appears that the virus reaches the liver by way of the blood stream. Undiluted blood and serum from intracerebrally inoculated hamsters collected at 24 and 44 hours killed all recipients when inoculated intracerebrally in 46 to 48 and 24 to 36 hours respectively, with typical liver lesions. All members of a similarly inoculated group, but receiving immune horse serum intraperitoneally, survived. These experiments demonstrate that viremia is present in the hamster infected with equine abortion virus. The mechanism of the viremia is under investigation.

\* Aided by a grant from the Grayson Foundation.



**A STUDY OF THE PATHOGENESIS OF THE VIRUS HEPATITIS OF MICE (NELSON'S)  
WITH SPECIAL REFERENCE TO MORPHOLOGIC CHANGES AND VIRUS TITER.**

Bernard F. Fetter (by invitation), Department of Pathology, Duke University School of Medicine, Durham, N.C.

*Abstract.* Forty PRI mice were inoculated by the intraperitoneal route with a 1 to 10 suspension of liver in saline solution and killed at various intervals thereafter. At 6 hours an occasional group of mononuclear cells appeared in the midzonal areas of the liver. Eighteen hours later the first evidence of necrosis and neutrophilic reaction was seen, this also in the midzonal area. The process of necrosis and neutrophilic reaction increased in intensity to the 3rd or 4th day. No fresh necrosis was recognized after the 5th day. On the 7th day scarring of the liver and proliferation of liver cells had begun. By the 14th day the remaining necrotic liver cells had become mineralized and were phagocytosed by macrophages. The first morphologic changes of necrobiosis were an accumulation of hematoxylin-positive bodies in the cytoplasm followed by, or occurring concurrently with, an eosinophilia of the cytoplasm. No parallel changes in the number or distribution of the mitochondria were noted. This was followed by karyorrhexis or pyknosis of the nucleus. When the livers were removed for sectioning, portions were suspended in saline solution for infectivity studies. The maximal amount of virus was found to be present at 2 days. There was a latent period between maximal virus level and maximal morphologic cellular alteration of 2 days.

**FULMINANT CARBON TETRACHLORIDE POISONING.** Robert B. Jennings (by invitation) and William B. Wartman, Pathology Department, Northwestern University Medical School, Chicago, Ill.

*Abstract.* This study is based on 8 cases of fatal carbon tetrachloride poisoning with special emphasis on 4 patients who died less than 96 hours after exposure to this toxin. This fulminant course is unusual, as almost all patients, except for a small number who succumb to the anesthetic effects of this compound, die some time after the fourth day. The fulminant cases differ further from the usual cases of carbon tetrachloride poisoning in that the liver is the seat of massive necrosis and fat infiltration. Except for scattered intact parenchymal cells in the periportal areas, no viable liver cells are present. Also, a striking type of fatty change is present in the kidney where there are numerous large globules of subnuclear neutral fat in the proximal tubular cells. The pathologic findings in these 4 patients are contrasted with the findings noted in 3 patients with fatal carbon tetrachloride poisoning who lived 5, 10, and 13 days respectively. These patients had the typical pathologic picture of carbon tetrachloride poisoning with relatively small areas of centrilobular necrosis and regeneration in the liver, massive pulmonary edema, and the histologic picture of lower nephron nephrosis in the kidney.

**ADRENAL NECROSIS AND THROMBOSIS IN ROUTINE NECROPSIES.** Alfred Plaut, Winter Veterans Administration Hospital, Topeka, Kans.

*Abstract.* In a previous paper the relatively frequent occurrence of necrosis in the anterior pituitary body has been described. A corresponding investigation of the adrenal gland is reported. Adrenal necrosis has been found 13 times in 124 non-selected adult males. Older men predominated. In three fourths of the 124 cardiovascular and renal disease or cancer were the main findings. There were no infectious diseases, nor cases in which tuberculosis was the main diagnosis, except for one case of meningitis. There were 9 cases of hepatic cirrhosis and 10 with degenerative disease of the central nervous system. No significant correlation existed between the occurrence of necrosis and the underlying disease. Necrosis without

vascular lesion, corresponding to the pituitary necrosis, was found twice, once in a case of encephalomalacia, and once in a case of carcinoma of the pancreas with metastases. In 9 cases necrosis was found together with thrombosis of adrenal veins. The extent of thrombosis appeared sufficient for the causation of the necrosis in most cases. In one case of myelogenous leukemia there was much necrosis and little thrombosis. In one case a few thrombi were found, but no necrosis. No important changes in the walls of the adrenal veins were found. There was no significant thrombosis in other organs. In one case necrosis was found also in the anterior pituitary lobe. Adrenal necrosis may be more frequent today than it was in previous decades. The causes for this are obscure.

#### READ BY TITLE

**THE FIBRILLAR APPARATUS IN ALTERED CERVICAL EPITHELIUM.** Nicanor Machicao (by invitation) and James W. Reagan, Institute of Pathology, Western Reserve University, Cleveland, Ohio.

*Abstract.* This paper deals with a study of the fibrillar apparatus in the component cells of the stratified squamous epithelium of the uterine cervix in metaplasia, atypical hyperplasia, carcinoma *in situ*, and invasive cancer. The fibrillar apparatus includes (1) tonofibrils, (2) intercellular bridges, (3) the spiral fibers of Herxheimer, and (4) the concentric fibers of Eberth. These form an intercrossing, 3-dimensional system of intracellular and extracellular fibrils which support the cells.

This study is based on 34 selected cases of atypical hyperplasia, 25 of carcinoma *in situ*, and 33 cases of invasive carcinoma. Ten additional cases of atypical hyperplasia were studied. Advantage was taken of the uninvolved portion of these tissues to study the normal appearance of the fibrillar apparatus. This material was treated by the Regaud modification of the Heidenhain iron hematoxylin technique.

The fibrillar apparatus showed distinctive changes in altered cervical epithelium. In squamous metaplasia the fibrillar apparatus was represented by a few intercellular bridges, while tonofibrils and nodes of Bizzozero were not readily seen. Occasionally concentric fibers were observed at the periphery of the cells. In atypical hyperplasia the intercellular bridges were less numerous, the tonofibrils were indistinct, and in some instances were not identified. With more marked degree of atypicality the intercellular bridges were usually disrupted or absent and the spiral fibers of Herxheimer (normally scarce) were increased in number and observed at higher levels in the epithelium. In the small cell type of carcinoma *in situ* no nodes of Bizzozero, tonofibrils, or bridges could be demonstrated. The only fibrillar structures to be seen were the spiral fibers which appeared in considerable numbers. In the large cell type of carcinoma *in situ*, representing a well differentiated lesion, the fibrillar structures were not affected and showed a remarkable integrity. In invasive cancer the alterations were similar to those described for the small cell type of carcinoma *in situ*, with a notable increase in the number of spiral fibers which seem to replace the normal tonofibrils.

These tissue changes have a counterpart in isolated cells of cervical smears. This alteration of the fibrillar framework of the epithelium would explain quite satisfactorily the decreased mutual adhesiveness that permits neoplastic cells to shed readily and appear in large numbers in cervical smears.

**THE HISTOLOGY AND HISTOCHEMISTRY OF LESIONS PRODUCED IN RABBITS BY REPEATED INTRAVENOUS DOSES OF BOVINE GAMMA GLOBULIN.** J. F. A. McManus and (by invitation) W. S. Gilmer, Jr. and John Torbert, University of Virginia School of Medicine, Charlottesville, Va.

*Abstract.* Thirty-eight rabbits have been subjected to single or repeated injections



of solutions of bovine gamma globulin by the intravenous route. Seven control animals were studied. The organs have been examined histologically and in some instances histochemically for the presence of arterial, myocardial, and renal lesions. The most frequent lesion produced by intravenous injections of bovine gamma globulin in rabbits is an arteritis which resembles very considerably human periarteritis nodosa. The lesions in the myocardium show a number of resemblances to human rheumatic fever. A mucoid change in the valve cusps of the mitral valve of many rabbits following these injections, a rare auricular endocarditis, angle endocarditis, and occasional aortic valvulitis reinforce this similarity.

The glomerulitis or glomerulonephritis which occurs in rabbits following the injections of bovine gamma globulin is only superficially like that seen in the human. It is intercapillary in type and is most frequently associated with an arteritis.

The distortion of the kidney produced by pyelonephritis, by arteritis, and by glomerulitis with arteritis in the rabbit following intravenous injections of bovine gamma globulin solutions resembles that seen in human renal disease in that alkaline phosphatase is decreased in, or absent from, the proximal convoluted tubules in the injured areas of the kidney. A 5-nucleotidase concentration, such as is seen in human renal disease, has not been observed in the damaged glomeruli of the rabbit kidney under the conditions of this experiment. In the smooth muscle cells of the media of rabbit arteries 5-nucleotidase is normally present. A loss of this enzyme, somewhat more extensive than the histologic lesion, is seen in the arteries in the acute arteritis produced in this experiment. The 5-nucleotidase study of the arteries appears to be a very useful method for the study of experimental arteritis.

**QUANTITATIVE CYTOPATHOLOGY: WHAT DETERMINES THE SIZE OF THE NUCLEUS OF A CELL?** Robert C. Mellors, Pathology Laboratories and Division of Experimental Pathology, Memorial Center for Cancer and Allied Diseases, New York, N.Y.

*Abstract.* The answer to the question proposed in the title is being obtained by the interferometric analysis of the organic mass of resting and dividing nuclei. It is found that the organic mass of resting nuclei in somatic tissue (such as liver of the mouse) bears the relation  $2M:4M:8M$ , where  $2M$ , the organic mass of a diploid nucleus, is about  $30 \times 10^{-12}$  gm. The tetraploid value is  $4M$  and the octoploid,  $8M$ . When the organic mass of sets of chromosomes in dividing cells (germinal cells of the mouse testis) is measured, the relation  $2M:4M:8M$  is found again, where  $2M$ , the organic mass of a diploid set of chromosomes, is about  $30 \times 10^{-12}$  gm. Thus, the organic mass of the diploid nucleus is assignable to the two sets of chromosomes contained within it. Since there is relative constancy, as shown by micro-refractometry, in the concentration of organic mass (the quantity per unit volume) in the nucleus of cells, it follows that the nuclear volume (and in a sense the nuclear area) is primarily determined by the organic mass within it, and this in turn is generally accounted for by the numbers of chromosome or chromatid structures within the nucleus. Of course, in degenerative changes accompanied by pyknosis or hydropic infiltration of the nucleus the foregoing relations do not prevail, and in certain specialized cells, such as motor neurons, it may be found, as suggested by others, that the assignment of nuclear organic mass must be made not alone to chromosomal, but also to extrachromosomal, structures.

**EARLY CHANGES IN THE MOUSE KIDNEY AFTER EXPERIMENTAL BURN SHOCK. I. FINDINGS IN UNTREATED AND SALINE TREATED MICE.** Robert W. Mowry and (by invitation) R. Carl Millican, University of Alabama Medical Center, Birmingham, Ala., and the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

*Abstract.* Much attention has been given to methods for the production of hemoglobinuric nephrosis (lower nephron nephrosis, etc.) in experimental animals.

Most require hemoglobin injection and other measures bearing little resemblance to circumstances leading to hemoglobinuric nephrosis in man. Thermal burn is a common type of injury and not infrequently leads to hemoglobinuric nephrosis. Rosenthal (1942) described a simple method for standardized burn injury in mice. Shaved mice are partly immersed in water at 70° C. for 5.5 seconds. Most of the mice develop a shock-like state within several hours and 90 to 100 per cent die before 24 hours. Fluid therapy reduces mortality. One ml. of 0.9 per cent NaCl given intravenously 1 hour after injury reduces 24-hour mortality to about 50 per cent.

Mice subjected to experimental burn shock frequently develop gross hemoglobinuria. Both dying and serially sacrificed mice were necropsied at intervals of 1 to 24 hours after injury. Thin slices of kidney were fixed for about 2 days in neutral buffered 10 per cent formalin and mordanted for 24 hours in 3 per cent potassium dichromate. Paraffin sections were stained by the azure-eosin method prescribed by Lillie. Hemoglobin is colored bright orange-red and is easier to recognize after azure-eosin staining than in hematoxylin-eosin preparations. Various more specific methods for hemoglobin also were used, including Lison's leuko-patent blue reaction. Paraffin sections of cold alcohol-fixed tissues were tested for alkaline phosphatase by Gomori's method.

One to 2 hours after the burn, the kidneys from most mice showed hemoglobin in the lumen and cells of the proximal convoluted tubules. Within the nephrons, cast formation by hemoglobin was not observed except in the distal convoluted and collecting tubules. Here the lumina were often plugged by homogeneous, deeply acidophilic masses giving a positive leuko-patent blue reaction. Hemoglobin casts were numerous in about half of the mice examined. By 4 to 6 hours after the burn, about 70 per cent of the fatal cases showed numerous hemoglobin casts in the distal renal tubules. Patchy necrosis in the broad limb of Henle was sometimes noted. Rarely, extensive necrosis of Henle's loops occurred. There was usually profound hyperemia of the vasa rectae in the medulla. Hemoglobin resorption by cells of the proximal convoluted tubule was still present but associated with vacuolization of the epinuclear cytoplasm. After 12 hours, the deeply acidophilic droplets believed to be hemoglobin were inconspicuous in the cells of the proximal tubules, but hydropic vacuolization of the epinuclear cytoplasm was more advanced. Most of the animals in the untreated group died. Many saline-treated mice still lived, but on sacrifice showed abundant hemoglobin casts in the majority of specimens. Renal epithelial cells around hemoglobin casts showed little or no change. The amount and distribution of alkaline phosphatase in the renal tubules was not abnormal within the period studied. Saline therapy (1 ml. of 0.9 per cent NaCl intravenously) did not materially reduce hemoglobin-cast formation or significantly modify the changes. Non-protein nitrogen values in saline-treated mice were about three times normal when determined at 10 and 24 hours after injury. It is concluded that the method produces renal damage resembling that seen after extensive burns in humans and may be useful in the experimental study of hemoglobinuric nephrosis.

**INCREASED RESISTANCE TO MALARIA IN CERTAIN INBRED MICE, THEIR HYBRIDS AND BACKCROSSES.** E. M. Nadel, J. Greenberg, G. E. Jay, and G. R. Coatney (all by invitation), National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Md.

*Abstract.* Studies on interrelationships between malaria and leukemia in mice disclosed significant differences in the resistance of DBA/2 and C57BL/6 mice and their F<sub>1</sub> hybrids to malaria. Ten additional strains from the Inbred Nucleus of the National Institutes of Health (A/L, SWR, C<sub>3</sub>H, STR, BRSUNT, C<sub>58</sub>, BALB/c, C57BR/cd, AKR, and C57L) and hybrids from these strains were studied for the presence of increased hybrid vigor in respect to resistance to malaria. In 45 series utilizing 375 mice, C57BL/6 and C57L animals survived significantly longer than all other strains tested. In 35 series utilizing 322 mice hybrids of C57BL/6 or of

C57L, animals survived significantly longer than all other inbred strains and all other hybrids tested with the exception of their mutual hybrid (C57BL/6 x C57L) which was less resistant than either parent. The shortest lived strain (A/L) survived a mean of 8.4 days, and the longest lived strain (C57BL/6), 17.6 days. Within each strain, older and female animals lived slightly longer than younger and male animals. In backcross studies, offspring resulting from the mating of the resistant parent strain and its more resistant hybrid, lived significantly longer than offspring of the same hybrid and the less resistant strain.

PROPAGATION IN VITRO OF EQUINE ABORTION VIRUS IN HUMAN EPITHELIAL CELLS.

(STRAIN HELA, GEY—CARCINOMA OF CERVIX.)\* Charles C. Randall, Department of Pathology, Vanderbilt University School of Medicine, Nashville, Tenn.

**Abstract.** The well known tissue culture strain of Dr. George Gey, designated HeLa, derived from a squamous cell carcinoma of the human cervix, was used in the study. The cell strain appears to be purely epithelial and because of ease of manipulation and subculture it has proved to be an admirable system for virus studies. A number of virus agents of man have been propagated in this type cell by other workers (Syvertson and Scherer). Our efforts to cultivate the virus of equine abortion in fetal horse tissue *in vitro* led us to the utilization of this cell strain which has been maintained on the naked glass surface of Carrel flasks and serum bottles with ascitic fluid as nutrient. The tissue cultures were inoculated with proved equine abortion virus, referred to above. In summary, the experimental data show that a virus (absent in control cultures) has been propagated in serial passage through human epithelial cells with the production of intranuclear inclusions. Passage virus, when inoculated into fetal horse tissue maintained by the Maitland method, showed similar intranuclear lesions. The evidence supports the claim that the virus in question is that of equine abortion. Final proof awaits evaluation of serologic studies.

TUBEROUS SCLEROSIS AND SPLENOMEGALY WITH FOCAL ACCUMULATIONS OF STORAGE CELLS, WITH ASSOCIATED TUMORS OF THE RETINA AND NODULAR GLYCOGENIC TUMORS OF THE HEART. G. Young, I. Young, N. W. Winkelman (all by invitation), and H. Brody, University of Pennsylvania Graduate Hospital and Albert Einstein Medical Center, Northern Division, Philadelphia, Pa.

**Abstract.** A male infant, the third child of a 30-year-old woman with two previous normal deliveries, was cyanotic at birth, in respiratory distress, and died at 4 hours. Necropsy was performed 7 hours post mortem. A normal appearing male infant measured 47 cm. and weighed 3,000 gm. The heart weighed 25.5 gm. (normal, 20 gm.). It showed multiple small glycogenic tumors, involving the myocardium of both ventricles. The spleen was enlarged, weighing 18 gm. (normal, 10 gm.). In scattered foci throughout the pulp were found collections of large cells with faintly eosinophilic cytoplasm, occasionally vacuolated. They suggested storage cells. Sudan IV stain revealed no fat; Best's stain for glycogen was negative; the Hotchkiss-McManus periodic acid-Schiff reaction was positive. In the eye, ganglionic cell tumors were found in the retina. The brain showed typical lesions of tuberous sclerosis.

In a review of the literature we have not found a case with similar involvement of the spleen. Although a few authors have mentioned splenic tumors, none of these has been documented with histologic descriptions or microphotographs. The findings in this case raise the possibility that cases of tuberous sclerosis with glycogenic nodular tumors in the heart may be an expression of a basic metabolic disturbance, and may be related, in a sense, to the group of metabolic storage diseases such as Tay-Sachs', Niemann-Pick's, and Gaucher's diseases.

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